Pharmacological reversal of cognitive bias in the chick anxiety-depression model

Kristen A. Hymel a, *, Kenneth J. Sufka a,b,c

a Department of Psychology, University of Mississippi, Oxford, MS 38677, USA
b Department of Pharmacology, University of Mississippi, Oxford, MS 38677, USA
c Research Institute of Pharmaceutical Sciences, University of Mississippi, Oxford, MS 38677, USA

Abstract

Cognitive bias presents in clinical populations where anxious individuals adopt a more pessimistic interpretation of ambiguous aversive stimuli and depressed individuals adopt both a more pessimistic interpretation of ambiguous aversive stimuli and a less optimistic interpretation of ambiguous appetitive stimuli. These biases have been reversed by anxiolytics and antidepressants. In the current study, chicks exposed to an isolation stressor of 5-min to induce an anxiety-like state or 60-min to induce a depressive-like state were tested in a straight alley maze to a series of morphed ambiguous appetitive (chick silhouette) to aversive (owl silhouette) cues. Chicks in the depression-like state displayed more pessimistic-like and less optimistic-like approach behavior to ambiguous aversive and appetitive cues, respectively. Both forms of cognitive bias were reversed by 15.0 mg/kg imipramine. Chicks in anxiety-like state displayed more pessimistic-like approach behavior under the ambiguous aversive stimulus cues. However, 0.10 mg/kg clonidine produced modest sedation and thus, was ineffective at reversing this bias. The observation that cognitive biases of more pessimism and less optimism can be reversed in the depression-like phase by imipramine adds to the validity of the chick anxiety–depression model as a neuropsychiatric simulation.

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1. Introduction

Anxiety and depression are common and debilitating clinical disorders. While many patients show clinical improvement with anxiolytics and antidepressants, these drugs may produce unpleasant side effects, and a significant number of patients are unaffected by current pharmacotherapeutic options (Davidson and Connor, 2004; Krishnan, 2004; Nelson, 2004; Rosenbaum and Tollefson, 2004). Advancements in novel pharmacotherapies for psychiatric disorders rely, in part, on the development, validation and utilization of animal model simulations. While the elevated plus maze and the forced swim test, are common models of anxiety and depression in behavioral pharmacology, respectively, they are not without problems. (Frazer and Morilak, 2005; Kalueff and Tuohimaa, 2004; Kalueff et al., 2007).

The chick anxiety–depression model (Sufka et al., 2006), which simulates both clinical syndromes within a single paradigm, may be a useful adjuvant to traditional models. The procedure involves a social separation stress that initially produces high distress vocalization (DVoc) rates characteristic of an anxiety-like state (i.e., panic model; Pansepp et al., 1978, 1980; Warnick et al., 2006) that is followed by lower DVoc rates characteristic of a depression-like state (i.e., behavioral despair model; Lehr, 1989). These phases can be pharmacologically dissociated in that diverse compounds possessing anxiolytic effects (e.g., chlordiazepoxide, clonidine and imipramine) attenuate the high DVoc rates during the anxiety-like phase while compounds possessing antidepressant effects (e.g., imipramine, maprotiline and fluoxetine) attenuate the reduction in DVoc rates during the depression-like phase (Sufka et al., 2006; Warnick et al., 2009; see also Lehr, 1989). Additionally, common stress and depression biomarkers are present in the model and include elevated corticosterone and interleukin-6 (IL-6) levels (Sufka et al., 2006; Warnick et al., 2009). Further, the chick model has outperformed traditional depression models by avoiding two false positives (memantine and antalarmin) (Sufka et al., 2009) which were initially detected as efficacious in rodent screening assays (Nielsen et al., 2004; Kos and Popik, 2005) but not in clinical populations (Zarate et al., 2006; Schechter et al., 2005). The validity of any animal model simulation is based on how well that model fits the human clinical syndrome in terms of etiology, symptomatology, pathophysiology and response to treatments (van der Staa, 2006). One clinical feature of anxiety and depressive disorders is a disturbance in cognition called cognitive...
bias. More specifically, anxious individuals make more pessimistic judgments of ambiguous stimuli; depressed individuals not only make more pessimistic judgments of ambiguous stimuli, but also make less optimistic judgments of ambiguous stimuli (Wright and Bower, 1992; MacLeod and Byrne, 1996; Miranda and Mennin, 2007). Cognitive bias is observed on a wide variety of tasks that include interference tasks (e.g., a modified version of the Stroop Task), attentional probe tasks and homophone tasks, among others (for review see, Mathews and MacLeod, 1994; Mogg et al., 2006; Mogg and Bradley, 2005).

Recent research has demonstrated cognitive bias in the chick anxiety–depression model (Salmeto et al., 2011). Chicks exposed to an isolation stressor of 5-min to induce an anxiety-like state or 60-min to induce a depressive-like state were then tested in a straight alley maze to a series of morphed ambiguous appetitive (chick silhouette) to aversive (owl silhouette) cues. In non-isolated controls, runway start and goal latencies generally increased as a function of greater amounts of aversive characteristics in the cues. In chicks in the anxiety-like state, runway start latencies increased to ambiguous aversive cues, reflecting more pessimistic-like behavior. In chicks in the depression-like state, runway start latencies increased to both aversive and appetitive ambiguous cues, reflecting more pessimistic-like and less optimistic-like behavior, respectively. The observation that cognitive biases in the chick anxiety–depression model are homologous to that of the human clinical syndromes adds an important validation step of the model as a neuropyschiatric simulation.

Several studies in clinical populations have shown cognitive bias reversed by various antidepressant drug classes. Anxious individuals given a serotonin selective reuptake inhibitor (SSRI) showed a reversal of negative interpretation bias on threat-related cues (i.e., more pessimism) on the modified Stroop Task (Weinstein and Nutt, 1995) and on the homophone task (Mogg et al., 2004). Depressed individuals given a norepinephrine selective reuptake inhibitor (NSRI) showed a reversal of decreased ability to recognize happy expressions (i.e., less optimism) on a facial recognition task and a reversal of negative bias to positive self-referential characteristics on an emotional categorization and memory task (Harmer et al., 2009). Interestingly, in anxious individuals, benzodiazepine (BZ) anxiolytics do not affect cognitive bias on the modified Stroop Task. This has been attributed to cognitive slowing that accompanies BZ receptor agonism (Golombek et al., 1991; Stewart et al., 2000).

The present research sought to determine whether the patterns of cognitive bias in the chick anxiety–depression model are similarly sensitive to pharmacological reversal. If such a homology exists, antidepressant administration should a) reverse more pessimistic-like behavior under ambiguous aversive cues in both anxiety-like and depression-like states and b) reverse less optimistic-like behavior under ambiguous appetitive cues in the depression-like state. It is also possible that a non-benzodiazepine anxiolytic may reverse more pessimistic-like behavior in the anxiety-like state. Such findings would further validate the chick anxiety–depression model as a neuropsychiatric simulation.

2. Methods

2.1. Subjects and housing characteristics

Cockerels (Gallus gallus; W38; Cal-Maine Foods, Inc., Mendenhall, Mississippi, USA) were received 1-day post hatch and housed in 34 × 57 × 40 cm stainless steel cages with 12–13 chicks per cage. Chicks were removed and briefly handled daily to minimize experimenter-related stress. Food (Purina Start and Grow, St Louis, Missouri, USA) and water was available ad libitum through one quart gravity-fed feeders (Murray MacMurray; Model 4BGF) and waterers (Murray MacMurray; Model 4QWD). Room temperature was maintained at 29 ± 1 °C and overhead illumination was maintained on a 12-h light–dark cycle (7 am–7 pm).

2.2. Apparatus

2.2.1. Straight alley maze

The apparatus consisted of a 50 × 30 × 10 cm arena made of opaque high-density polyethylene material that contained a straight alley maze adjacent to a holding arena (see Salmeto et al., 2011 for full description). Briefly, the maze consisted of a 10 × 10 cm start box with a guillotine door that opens up to a 40 × 10 cm runway with either an 8 × 10 cm mirror or various 8 × 10 cm stimulus places at its end. A 40 × 20 cm holding arena housed 12 conspecifics throughout the test session and permitted the testing of chicks under non-isolated treatment conditions. These conspecifics remained out of view during maze testing. However, once chicks reach the goal, full view of the arena was permitted through a 20 × 10 cm clear Plexiglas wall. Pine bedding was placed throughout the arena and food and water was available ad libitum in 200 ml stainless steel cups.

2.2.2. Morphed stimulus conditions

Morphus Photo Morpher v3.01 Professional for Mac (Morphus Software, LLC) was used to produce ‘morphed’ images that blended elements of a chick and a horned owl silhouette by matching a series of approximately 200 dots onto each photo to match the location of the dots between the images. This allowed for 100 morphed frames linking the start (chick) and end (owl) photos. Within this series two key frames were defined: 75% chick and 25% owl, and 25% chick and 75% owl were used (75%250 and 25%750). The pixelated edges of the images were smoothed out and the images were adjusted so that they were all approximately the same size and fit on an 8 × 10 cm stimulus card. The images were saved as jpeg files, printed and placed behind a clear glass plate during testing (see Salmeto et al., 2011 for pictures of morphed stimuli).

2.3. Isolation apparatus

A six-unit test apparatus containing Plexiglas viewing chambers (25 × 25 × 22 cm) situated in sound-attenuating enclosures was used to collect isolation-induced distress vocalizations. The units were illuminated using 25 W light bulbs and ventilated by an 8-cm diameter rotary fan (Model FP–IIBAX51; Rodale, Great River, New York, USA). Miniature video cameras (Model PC60XP; Super Circuit, Liberty Hill, Texas, USA) mounted at floor level in the corner of the enclosures and routed through a multiplexer (Model PC47MC; Super Circuit) allowed for animal observation. Distress vocalizations were collected via microphones [Model 3-675-001 (modified); Lafayette Instruments, Lafayette, Indiana, USA] mounted on the rear wall of the Plexiglas chambers, routed through sound-activating relays (Model 630400A; Lafayette Instruments; settings: 60–75% sensitivity, 0.10-s delay) and collected a USB interface via custom-designed software.

2.3. Pilot study

The notion that anxiolytic sedative effects could confound runway performance, a pilot study determined optimal dosing for clonidine and imipramine under non-isolated test conditions. The pilot study revealed one unexpected outcome: exposure to the test protocol in non-isolated groups led to modest but measurable and pharmacologically dissociable stress behaviors on DVOs and runway performance under ambiguous stimulus cues. This stress effect is likely due to experimenter exposure, weighing and injection procedures, apparatus novelty and flock reduction associated with the test protocol (Feltenstein et al., 2002). These findings prompted the use of a no-test group (i.e., no exposure to isolation test apparatus prior to maze testing) to serve as the control for the experiment.

2.4. Procedure

This experiment was conducted to test whether cognitive bias could be reversed under an anxiety-like and a depression-like state. In the first trial, at age 4 days post hatch, 12 cagemate conspecifics were placed into the holding arena and individually tested in the maze under the mirror cue condition. Each chick was placed into the start box for 15-s after which the guillotine door was raised. Dependent measures were start and goal latency and farthest distance traveled. Start latency was defined as the time the chick took to step completely outside the start box. Because all test sessions were terminated at 5-min, the farthest distance traveled (cm) from the start box was measured to account for possible differences between chicks that complete the maze and those that did not. Chicks were placed back into the holding arena until all were tested. Group assignment for Trial 2 was based on goal latency performance in Trial 1.

In the second trial, at either 5 or 6 days post hatch, chicks were administered either 0.10 mg/kg clonidine (tested for 5-min), 15.0 mg/kg imipramine (tested for 60-min), a physiological saline (tested for 5-min), and a physiological saline (tested for 60-min). All chicks were injected with drug probes 15-min prior to testing. Following apparatus testing, chicks were transported from the isolation apparatus in a 2-quart opaque plastic container and tested immediately in the maze under one of four stimulus cue conditions: mirror, 75%250 morph, 25%750 morph: or 0:100 (owl silhouette). To assess a baseline for each stimulus cue, a no-isolation test control group was administered physiological saline and tested immediately within the maze. In addition, these chicks remained in the arena throughout the test
session. Dependent measures for the maze were start latency and distance traveled; previous research demonstrated that the goal latency measure does not reveal cognitive bias under ambiguous cues due to ceiling effects imposed by the 5-min test criterion (Salmeta et al., 2011). Chicks were returned to their home cage after testing. All efforts were made to minimize animal suffering and to reduce the number of animals used. All procedures were approved by the University of Mississippi Institutional Animal Care and Use Committee (protocol 10-006).

2.5. Statistical analysis

To assess for significant isolation group differences and significant pharmacological effects on distress vocalizations in the anxiety-like and depression-like phases, all DVocs were transformed into a rate/min function. A one-way ANOVA was conducted on the anxiety-like phase (i.e., first 5-min/5) and a t-test was conducted on the depression-like phase (i.e., 30-60-min/30). Post-hoc analyses for the anxiety-like phase were conducted using Fisher’s least significant difference tests.

To assess for significant isolation group differences and significant pharmacological effects on the cognitive biases seen under each individual stimulus cue, four 2 × 5 MANOVAs were conducted with mean start latency and mean distance traveled as the dependent variables. A priori planning to assess group differences across each stimulus cue individually set the MANOVA p-value at p < 0.0125. Given the significance of the MANOVA, a one-way ANOVA was conducted upon each dependent variable. Given the significance of the ANOVA, a Tukey’s HSD post-hoc analysis was conducted to compare group means of the five drug treatment conditions. Chicks that were clearly sedated (i.e., adopted a ventral recumbent posture with drooped head and eyes closed) within the maze were discarded from the analysis.

3. Results

3.1. Distress vocalizations

The effects of stress × drug treatment conditions on DVoc rates for chicks tested in the anxiety-like and depression-like phases are presented in Fig. 1A and B, respectively. Chicks in the vehicle group displayed relatively high DVoc rates in the first 5-min, indicative of an anxiety-like state; DVoc rates declined by approximately 50% of the initial response rate for the final 30-min of the test session indicative of a depression-like state (i.e., behavioral despair). In the anxiety-like phase, clonidine and imipramine groups displayed DVoc rates that were attenuated compared to the vehicle group. In the depression-like phase, the imipramine group displayed DVoc rates that were higher compared to the vehicle group.

Consistent with these observations, a one-way ANOVA conducted on the anxiety-like phase revealed a significant main effect for Treatment \( F(2,195) = 42.43, p < 0.005 \). In the anxiety-like phase (see panel A), Fisher’s LSD revealed that the clonidine and imipramine groups displayed significantly lower mean DVoc rates compared to the vehicle group (ps < 0.005). A t-test conducted on the depression-like phase revealed a significant effect for Treatment \( t(100) = 4.28, p < 0.001 \), where the imipramine group displayed significantly higher DVoc rates compared to the vehicle group (see panel B).

3.2. Runway performance × stimulus cues

3.2.1. Mirror stimulus cue

The effects of stress × drug treatment conditions on runway performance under the mirror stimulus cue are presented in Fig. 2. Start latencies (panel A) for all groups were short with one exception. The vehicle-depression group had a longer start latency compared to the vehicle no-test group (i.e., less optimism) and this effect was reversed by imipramine. No detectable group differences were observed for distance traveled (panel B).

Consistent with these observations, a one-way MANOVA revealed a significant main effect for treatment, Wilks’ \( \lambda = 0.606, F(8, 120) = 4.266, p < 0.001 \), where \( p < 0.0125 \) is considered significant, partial eta squared = 0.221. Power to detect the effect was 0.993. Given the significance of the MANOVA, univariate main effects were examined. Significant main effects for treatment were obtained for start latency \( F(4,61) = 7.909, p < 0.001 \) and distance traveled \( F(4,61) = 2.860, p < 0.05 \). Tukey’s HSD revealed significantly longer mean start latency for the vehicle-depression group compared to the vehicle no-test group and a significantly shorter mean start latency for the imipramine-depression group compared to the vehicle-depression group (ps < 0.005). Tukey’s HSD did not reveal any statistically significant group differences for mean distance traveled.

3.2.2. 75c:25o (ambiguous chick stimulus cue)

The effects of stress × drug treatment conditions on runway performance under the 75c:25o stimulus cue are presented in Fig. 3. Once again, start latencies (panel A) were under 1-min in the vehicle no-test group. In general, start latencies were longer among isolation groups. The vehicle-depression group presented the longest start latency compared to the vehicle no-test group (i.e., less optimism), and this effect was reversed by imipramine. Consistent with these observations, a one-way MANOVA revealed a significant main effect for treatment, Wilks’ \( \lambda = 0.617, F(8,112) = 3.817, p < 0.005 \), partial eta squared = 0.214. Power to detect the effect was 0.985. Given the significance of the MANOVA, univariate main effects were examined. A significant main effect for treatment was obtained for start latency \( F(4,57) = 7.428, p < 0.001 \). Tukey’s HSD revealed significantly longer mean start latency for the vehicle-depression group compared to the vehicle no-test group and a significantly shorter mean start latency for the imipramine-depression group compared to the vehicle-depression group (ps < 0.001).
Distance traveled (panel B) was 25-cm for the no-test group. In general, distance traveled was shorter among the isolation groups. The vehicle-depression group presented the shortest distance traveled compared to the vehicle no-test group (i.e., less optimism) and this effect was reversed by imipramine. A significant univariate main effect for treatment was obtained for distance traveled ($F(4,57) = 7.261$, $p < 0.001$. Tukey’s HSD revealed a significantly shorter mean distance traveled for the vehicle-depression group compared to the vehicle no-test group and a significantly longer mean distance traveled for the imipramine-depression group compared to the vehicle-depression group ($p < 0.005$).

3.2.3. 25c:75o (ambiguous owl stimulus cue)

The effects of stress x drug treatment conditions on runway performance under the 25c:75o stimulus cue are presented in Fig. 4. As before, start latencies (panel A) were under 1-min in the vehicle no-test group. In general, vehicle isolation groups presented longer start latencies compared to the vehicle no-test group (i.e., more pessimism). The change in runway performance in the depression group was reversed by imipramine. Consistent with these observations, a one-way MANOVA revealed a significant main effect for treatment, Wilks’ $\lambda = 0.596$, $F(8, 104) = 3.383$, $p < 0.005$, partial eta squared = 0.228. Power to detect the effect was 0.985. Given the significance of the MANOVA, univariate main effects were examined. A significant main effect for treatment was obtained for start latency ($F(4, 53) = 6.824$, $p < 0.001$. Tukey’s HSD revealed a marginally significantly longer mean start latency for the vehicle-anxiety group compared to the vehicle no-test group ($p = 0.092$). The analysis also revealed a significantly longer mean start latency for the vehicle-depression group compared to the vehicle no-test group ($p < 0.001$) and a significantly shorter mean start latency for the imipramine-depression group compared to the vehicle-depression group ($p < 0.05$).

Distance traveled (panel B) was approximately 21-cm for the vehicle no-test group. In general, distance traveled was shorter among the isolation groups. The vehicle-depression group presented the shortest distance traveled compared to the vehicle no-test group (i.e., more pessimism), and this effect was reversed by imipramine. A significant univariate main effect for treatment was obtained for distance traveled ($F(4, 53) = 4.217$, $p < 0.01$. Tukey’s HSD revealed a significantly shorter mean distance traveled for the vehicle-depression group compared to the vehicle no-test group ($p < 0.005$) and a significantly longer mean distance traveled for the imipramine-depression group compared to the vehicle-depression group ($p < 0.05$).

3.2.4. 0c:100o (owl stimulus cue)

The effects of stress x drug treatment conditions on runway performance under the 0c:100o stimulus cue are presented in Fig. 5. As before, start latencies (panel A) were under 1-min in the vehicle no-test group. Vehicle isolation groups presented longer start latencies compared to the vehicle no-test group (i.e., more pessimism). The change in runway performance in the depression group was reversed by imipramine. One unexpected outcome was a longer start latency in the clonidine-anxiety group and we believe this was due to sedation. Consistent with these observations, a one-way MANOVA revealed a significant main effect for treatment, Wilks’ $\lambda = 0.659$, $F(8, 114) = 3.303$, $p < 0.005$, partial eta squared = 0.188. Power to detect the effect was 0.966. Given the significance of the MANOVA, univariate main effects were...
Behavior was reversed by imipramine in the depression-like phase. Further, more pessimistic-like and less optimistic-like behavior on runway performance under ambiguous aversive cues, and chicks in the depression-like phase display both more pessimistic-like and less optimistic-like behavior on runway performance under ambiguous aversive cues, and chicks in the depression-like phase display reduced DVoc rates by up to 30% compared to the vehicle no-test group (Sufka et al., 2006). In addition, clonidine attenuates Dvos in the anxiety-like phase (Warnick et al., 2006), whereas imipramine prevents the onset of behavioral despair (Sufka et al., 2006; Warnick et al., 2009).

Consistent with previous findings from Salmeto et al. (2011), chicks in the anxiety-like phase display more pessimistic-like behavior on runway performance under ambiguous aversive cues, and chicks in the depression-like phase display both more pessimistic-like and less optimistic-like behavior on runway performance under ambiguous aversive and appetitive cues, respectively. Further, more pessimistic-like and less optimistic-like behavior was reversed by imipramine in the depression-like phase.

4. Discussion

Cognitive bias is a phenomenon that presents in individuals suffering from anxiety or depression in which cognitive disturbances elicit negative interpretations of ambiguous stimuli and/or events. Such biases have been reversed using anxiolytics and antidepressants (Harmer et al., 2009; Mogg et al., 2004; Weinstein and Nutt, 1995). Cognitive bias has previously been examined in the chick anxiety-depression model using a measure of approach/avoidant behavior to a range of appetitive to aversive stimulus cues in a straight alley maze (Salmeto et al., 2011). The demonstration of reversing the cognitive biases that presents under the anxiety-like and depression-like phases within the straight alley maze would provide further validation of the chick anxiety-depression continuum model as a neuropsychiatric simulation.

In the current study, the observed pattern of DVoc rates are consistent with previous studies wherein chicks in the anxiety-like phase initially produce high DVoc rates (i.e., first 5-min of isolation) and chicks in the depression-like phase display reduced DVoc rates (i.e., final 30–60-min of isolation) (Sufka et al., 2006). In addition, clonidine attenuates DVoc in the anxiety-like phase (Warnick et al., 2006), whereas imipramine prevents the onset of behavioral despair (Sufka et al., 2006; Warnick et al., 2009).
However, more pessimistic-like behavior was not reversed by clonidine in the anxiety-like phase; clonidine appeared to have sedative effects on runway performance. The results of the present study are consistent with findings that cognitive bias can be reversed by antidepressants in clinical populations (Harmer et al., 2009).

The inability of clonidine to reverse cognitive bias in the anxiety-like phase appears to be related to the sedative nature of this compound (Dahmani et al., 2010; Feltenstein et al., 2004; Warnick et al., 2006). While we excluded from data analyses chicks that were overly drowsy or fully asleep (i.e., adopted a ventral recumbent posture with drooped head and eyes closed), it is likely that in other instances runway behavior may have been slowed in moderately sedated chicks that did not fully meet this criterion for exclusion. Although this presented some statistical problems (e.g., smaller sample size, larger error variance, and insufficient power), runway behavior remains confounded and we are unable to draw conclusions on whether clonidine is capable of reversing cognitive bias. Other pharmacological options that may reverse cognitive bias in the anxiety-like state may include serotonin selective reuptake inhibitors (SSRIs) which have been shown to reverse cognitive bias in anxious individuals (Mogg et al., 2004; Weinstein and Nurt, 1995). However, benzodiazepines are not an ideal choice as they have been shown to produce sedative effects in human clinical populations (Golombek et al., 1991; Stewart et al., 2000) and in mice (Ennaceur et al., 2008).

Collectively, the observation that cognitive biases of both more pessimism and less optimism present within the single test paradigm of anxiety and depression and can be pharmacologically reversed in the depression-like phase adds to the validity of the chick anxiety–depression model as a neuropsychiatric simulation. The chick anxiety–depression model, along with the runway test to ambiguous appetitive and aversive cues, may lend itself to exploring the common neurophysiological mechanisms subserving cognitive disturbances and pharmacological responses seen in these two seemingly related clinical disorders.

References


