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Research Report

Cognitive bias in the chick anxiety–depression model

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ABSTRACT

Cognitive bias is a phenomenon that presents in clinical populations where anxious individuals tend to adopt a more pessimistic-like interpretation of ambiguous aversive stimuli whereas depressed individuals tend to adopt a less optimistic-like interpretation of ambiguous appetitive stimuli. To further validate the chick anxiety–depression model as a neuropsychiatric simulation we sought to quantify this cognitive endophenotype. Chicks exposed to an isolation stressor of 5 m to induce an anxiety-like or 60 m 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 latencies were increased to aversive ambiguous cues, reflecting more pessimistic-like behavior. In chicks in the depression-like state, runway latencies were increased to both aversive and appetitive ambiguous cues, reflecting more pessimistic-like and less optimistic-like behavior, respectively.

1. Introduction

Traditional animal models of anxiety and animal models of depression differ in their procedural manipulations and behavioral endpoint measures (Willner, 1991). However, a novel model using chicks that involves separation from conspecifics reveals both anxiety-like and depression-like behavior within a single paradigm on a single behavioral measure (Sufka et al., 2006). The chick anxiety–depression model involves social separation stress that initially produces high distress vocalization (DVoc) rates characteristic of an anxiety-like state (i.e., panic model; 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, 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

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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).

A recent study that screened the efficacy of seven compounds targeting novel CNS sites, each of which previously passed antidepressant screening in rodent models, yielded a somewhat different profile than the early preclinical screens. The chick anxiety–depression model identified prasterone, ketamine, mifepristone, CGP36742 and DOV216,303 as possessing antidepressant properties while memantine and antalarmin did not (Sufka et al., 2009). Interestingly, this pattern of effects is in line with early clinical trial outcomes and illustrates the predictive validity of the model by correctly detecting efficacy of five compounds and avoiding two false positives (Wolkowitz et al., 1999; Zarate et al., 2006a,b; Belanoff et al., 2002; Schechter et al., 2005). Collectively, these results not only question the predictive validity of rodent models, but also begin to provide support for the validity of the chick anxiety–depression model as a neuropsychiatric simulation/screening assay. However, the quality of a simulation increases with greater numbers of homologies demonstrated between the animal model and its clinical syndrome (Kalueff and Murphy, 2007; Miczek and de Wit, 2008; Panksepp, 2006; van der Staay, 2006).

Biases in cognitive function have been shown in anxious individuals who display more pessimistic judgments and in depressed individuals who display not only more pessimistic judgments, but also less optimistic judgments (Wright and Bower, 1992; MacLeod and Byrne, 1996; Miranda and Mennin, 2007). Pessimism is defined as an increase in the expectation of negative events whereas optimism is defined as a decrease in the expectation of positive events. An example of a more pessimistic cognitive style has been demonstrated in individuals diagnosed with generalized anxiety disorder (Mogg et al., 2004) and in those suffering from depression (Mogg et al., 2006) who reported a greater number of threat related responses to ambiguous homophones (e.g. die-dye, weak-week) compared to controls. An example of a less optimistic cognitive style has been demonstrated in depressed individuals undergoing treatment for metastatic renal cell carcinoma or metastatic melanoma who report significantly lower levels of treatment specific optimism (i.e., likelihood of being cured) compared to non-depressed matched controls (Cohen et al., 2001).

Cognitive biases related to altered affective states have been studied across a range of species including rhesus macaques, dogs, rats and birds (Harding et al., 2004; Burman et al., 2008; Bethell et al., 2007; Bateson and Matheson, 2007; Matheson et al., 2008; for reviews see Mendl et al., 2009; Brilot et al., 2010). Some of the most successful of these have required animals to learn that cues presented at opposite ends of a stimulus range (e.g., white vs. black) require approach and avoidant behavioral responses that are associated with appetitive (e.g., food) and aversive (e.g., white noise) outcomes, respectively. The animal is then exposed to a novel ambiguous stimulus cue (or cues) that fall within the original stimulus range. Responses to these ambiguous cues can be used to determine whether the animal expects a positive or negative event to occur. Exposure to stressors that impact emotional states is hypothesized to alter cognitive decision making in such tasks. For example, increased avoidant responses to ambiguous cues associated with a negative outcome reflect more pessimistic-like behavior. In contrast, decreased approach responses to ambiguous cues associated with a positive outcome reflect less optimistic-like behavior (see Fig. 1 for illustration).

Several recent studies have explored cognitive biases associated with stress states in avian models. For example, one study (Matheson et al., 2008) compared the performance of European starlings (*Sturnus vulgaris*) housed in chronic enriched versus impoverished cages on a temporal generalization task. The starlings were initially trained to discriminate two temporal stimuli (2 s vs. 10 s light cue) paired with instant versus delayed food reward, and were subsequently tested with ambiguous, intermediate-duration stimuli. The probability of classifying an intermediate stimulus as the stimulus associated with instant food (i.e. the better outcome) was lower in starlings housed in impoverished cages. This pattern was interpreted as reflecting reduced optimistic-like behavior associated with depression-like states induced by inadequate cage environments. More recent studies have explored the use of behavioral responses to ecologically-relevant stimuli (e.g., predator cues) that are likely to have been important in a species' evolutionary history (Brilot et al., 2009). The potential advantage of such stimuli lies in the reduced requirement for extensive associative training prior to the cognitive bias tests. The current study builds on this approach by using silhouettes of a conspecific chick (or mirror), an owl, and three intermediate ambiguous cues with varying degrees of chick and owl stimulus characteristics (see Fig. 2A). The two unmodified silhouettes are designed to possess a predetermined valence for a social prey species like domestic chicks: the Chick cue is predicted to be positive, whereas the owl (a potential predator) is predicted to be negative. To measure approach/avoidant responses we utilized a straight-alley maze (see Fig. 2B), a paradigm commonly used to quantify chick social reinstatement (Jones and Marin,

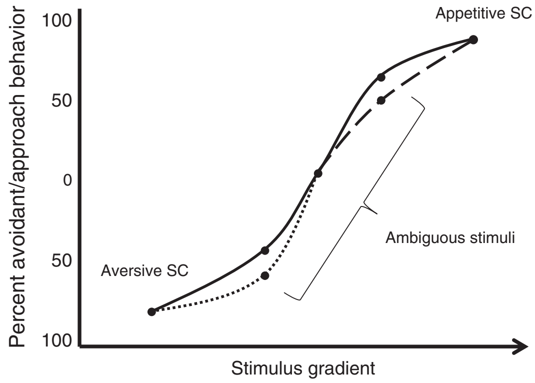

Fig. 1 – Predicted percent of avoidant/approach behavior to a range of stimulus cues (SC). In non-stressed control animals, predicted behavioral responses are indicated by the solid line. Dotted line represents increased avoidant behavior to ambiguous aversive cues and reflects more pessimistic-like behavior. Dashed line represents decreased approach behavior to ambiguous appetitive cues and reflects less optimistic-like behavior.

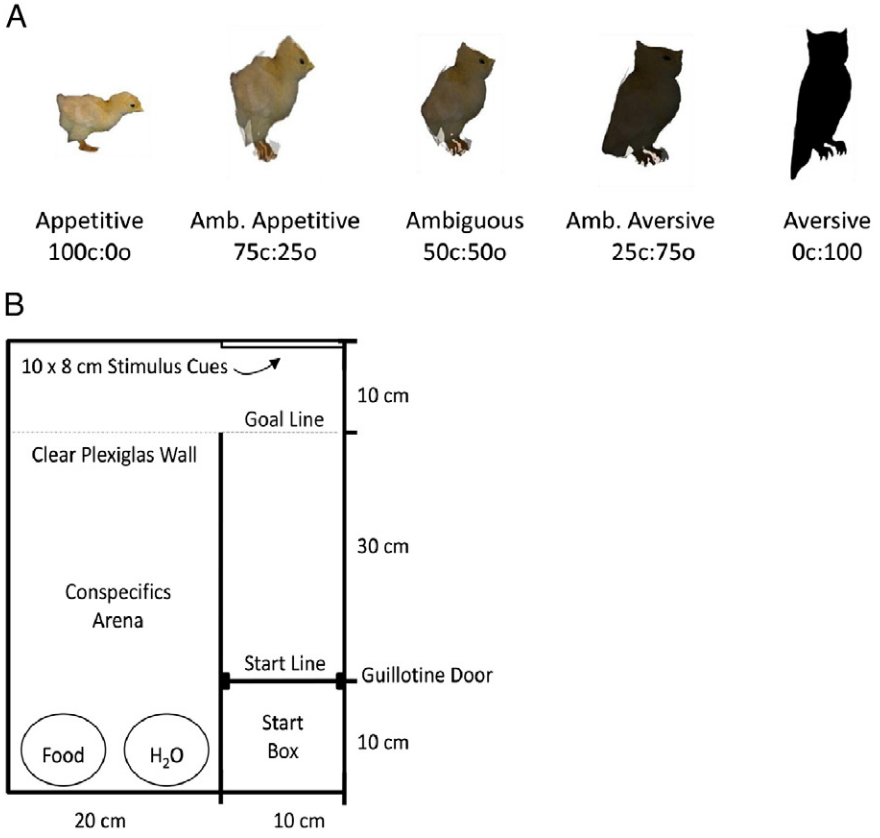

Fig. 2 – Panel A depicts stimulus cue conditions in straight alley maze. 100c = chick image and 100o = owl silhouette. The remaining images were derived via computer generated morphing software between these two images where 75c:25o, 50c:50o, 25c:75o represent silhouettes derived at 25%, 50% and 75% moving through the chick to owl morphing program, respectively. Panel B depicts a diagram of the straight alley maze apparatus.

1999; Marin and Jones, 2000; Marin et al., 2001; Clarke and Jones, 2001; Marin et al., 2003), but positioned various stimulus cues at the goal. We predicted that in non-stressed chicks, runway latencies would vary as a function of the degree of positive/negative valence components in the stimulus cues. In contrast, chicks tested under an anxiety-like state should demonstrate less approach behavior to ambiguous cues closest to the owl silhouette. Conversely, chicks tested under a depression-like state should demonstrate less approach behavior to ambiguous cues closest to both the chick and owl silhouettes. This pattern of cognitive bias would provide further validation of the chick anxiety–depression model as a neuropsychiatric simulation.

2. Results

2.1. *Experiment 1*

Runway start latencies for the five morphed stimulus conditions are summarized in Fig. 3A. Mean start latencies were relatively short (under 10 s) and did not significantly differ across conditions. Consistent with these observations, a 1-way ANOVA on these data failed to reveal a significant effect of stimulus cue, $F_{4,103} = 1.05$, $P =$ n.s. Runway goal latencies for these same 5 conditions are summarized in Fig. 3B. Mean goal latencies generally increased with greater amounts of owl silhouette in the stimulus morphs. Consistent with these observations, a 1-way ANOVA on these data revealed a significant treatment effect, $F_{4,102} = 6.28$, $P = 0.0001$. Fisher's post-hoc analyses revealed a significant increase in mean goal latency for the owl silhouette compared to all other stimulus conditions, $Ps < 0.05$. In addition, mean goal latency for the 25c:75o stimulus condition was significantly longer than that of the 75c:25o stimulus condition, $P < 0.05$. No other group comparisons were statistically significant.

2.2. *Experiment 2*

Runway start latencies for the five stimulus conditions under the Isolation treatment groups are summarized in Fig. 4A. Consistent with the findings of Experiment 1, mean start latencies in the Non-isolated groups across the stimulus conditions were relatively short (under 15 s). In general, mean start latencies in the Isolated-5 m and Isolated-60 m groups were much longer than the Non-isolated group. A priori planned comparisons predicted unique patterns of isolation group differences under each stimulus cue dependent upon the degree of its appetitive and aversive characteristics (Keppel and Wickens, 2004). In order to detect such group differences, five separate 1-way ANOVAs were conducted on these start latency data using a Bonferroni correction

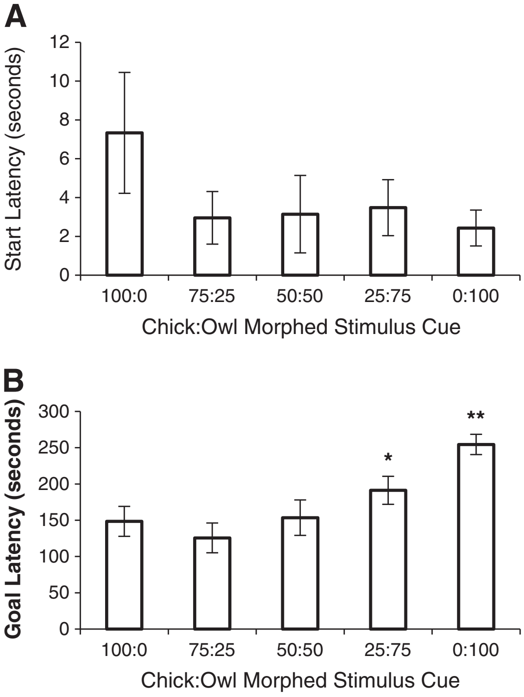

Fig. 3 – Mean start and goal latencies (+/−SEM) across stimulus cues in panels A and B, respectively. Percentages reflect amount of stimulus characteristics of chick:owl silhouettes (see Fig. 2B). These groups did not receive the isolation stress manipulation prior to maze testing. Sample sizes were $n=21–23$. *Indicates significant differences from 75c:25o cue. **Indicates significant difference from all other stimulus cue conditions.

procedure, which sets P-values at <0.01 for significant group differences. These analyses revealed an interesting pattern of effects. For example, under the mirror ($F_{2,38}=9.50$, $P=0.0005$) and 75c:25o ($F_{2,40}=8.08$, $P<0.005$) stimulus conditions, mean start latencies were significantly longer than the Non-isolated group for only the Isolated-60 m group ($Ps<0.0005$). In contrast, under the 50c:50o ($F_{2,39}=7.60$, $P<0.005$), 25c:75o ($F_{2,37}=10.09$, $P<0.0005$) and owl ($F_{2,39}=16.66$, $P<0.0001$) stimulus conditions, mean start latencies were significantly longer than the Non-isolated group for both the Isolated-5 m and Isolated-60 m groups ($Ps<0.05$). Finally, post-hoc analyses also revealed that under the 25c:75o and owl stimulus conditions, mean start latencies in the Isolated-60 m groups were significantly longer than in the Isolated-5 m groups, ($Ps<0.05$).

Runway goal latencies for the five stimulus conditions under the Isolation treatment groups are summarized in Fig. 4B. In general, goal latencies tended to be longer under stimulus conditions with greater amounts of owl silhouette in the cues and longer isolation intervals. As before, a priori planned comparisons predicted unique patterns of isolation group differences under each stimulus cue dependent upon the degree of its appetitive and aversive characteristics (Keppel and Wickens, 2004). In order to detect such differences, five separate 1-way ANOVAs were conducted on these goal latency data using a Bonferroni correction procedure, which sets P-values at <0.01 for significant group differences.

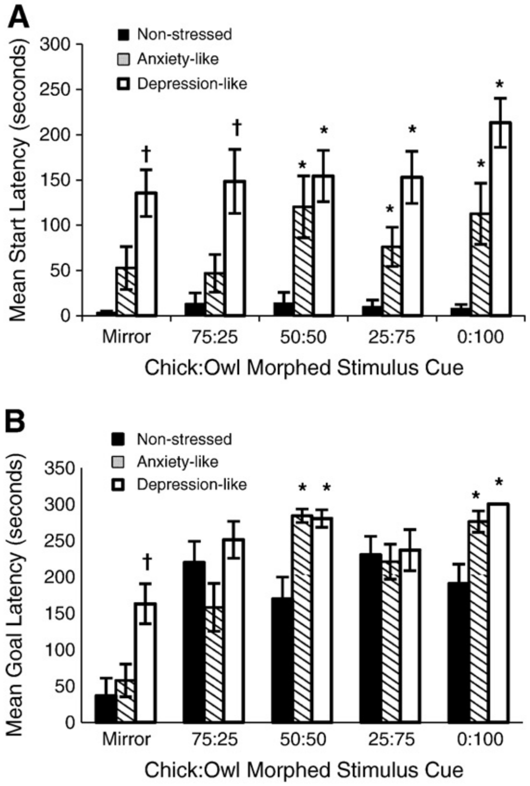

Fig. 4 – Mean start and goal latencies (+/−SEM) across stimulus cues and separated by Isolation treatment group in panels A and B, respectively. Percentages reflect amount of stimulus characteristics of chick:owl silhouettes. Non-stressed groups did not receive the isolation manipulation prior to maze testing. Anxiety-like groups received 5 m isolation prior to maze testing. Depression-like groups received 60 m isolation prior to maze testing. Sample sizes were $n=12–15$. *Indicates significantly longer latencies compared to the Non-isolated group under the aversive cues. †Indicates significantly longer latencies compared to the Non-isolated group under the appetitive cues.

These findings revealed a similar pattern of effects as the start latency analyses but only under three of the five stimulus conditions (ANOVAs for 75c:25o and 25c:75o=n.s.). Under the mirror stimulus condition ($F_{2,38}=7.51$, $P<0.005$), mean goal latencies in the Isolated-60 m group were significantly longer than both the non-isolated and Isolated-5 m groups ($Ps<0.005$). In contrast, under the 50c:50o ($F_{2,39}=11.07$, $P<0.0005$) and owl ($F_{2,39}=10.67$, $P<0.0005$) stimulus conditions, mean goal latencies were significantly longer than the Non-isolated group for both the Isolated-5 m and Isolated-60 m groups, ($Ps<0.05$).

3. Discussion

One prominent clinical feature of individuals suffering from anxiety and depression is biases in cognitive function. Changes in emotional states can lead to alterations in judgments of ambiguous stimuli that could be interpreted as either positive or negative (Eysenck et al., 1991). In other words, these cognitive biases present as either making more pessimistic-like or less optimistic-like judgments. The demonstration of such alterations in decision-making processes in the chick anxiety–depression model would further support its validity as a neuropsychiatric simulation.

In Experiment 1, approach–avoidant behavior to appetitive, aversive, and intermediate ambiguous and aversive stimulus cues were measured in non-isolated chicks using a straight alley maze. Start and goal latencies served as the dependent measure. Start latencies were unaffected by varying the stimulus cues. However, goal latencies were longer using stimuli with greater amounts of owl silhouette characteristics. These findings are consistent with other studies that have used naturally appetitive and aversive stimuli to produce approach and avoidant behavior, respectively. For example, runway speeds in chicks are faster when the goal box presents a video image and soundtrack of other chicks feeding (Clarke and Jones, 2001). In contrast, chicks avoid red and yellow colored crumbs, which are natural warning colors of unpalatable insects, in open field foraging tests (Rowe and Skelhorn, 2005). Similar avoidant behavior in feeding is seen in European starlings exposed to predator eyespot stimuli (Brilot et al., 2009). The graded responses seen under the ambiguous stimulus cues that varied in their appetitive and aversive characteristic ratios appear to provide the necessary range of stimuli with which to examine the possibility of cognitive bias under anxiety-like and depressive-like states. One additional benefit of using such naturally appetitive and aversive cues is that they elicit spontaneous approach and avoidant behavior, respectively. This stands in contrast to protocols employing color or tone cues that require large numbers of conditioning trials to elicit the same kind of behavioral responses (Brilot et al., 2010; Harding et al., 2004; Bateson and Matheson, 2007).

One unexpected finding was that the goal latencies under the chick stimulus cue (148.0 +/− 21.0 s, n=21) were much longer than the goal latencies under the mirror cue (27.6 +/− 5.0 s, n=102) in the pre-test session. Such longer latencies, under what we believed to be the most naturally appetitive cue, may be due to the absence of life-like characteristics of a still image. This interpretation suggests the most effective cue to stimulate the strongest approach behavior is something much more life-like and for this reason we decided to replace this Chick cue with a mirror in the second study.

The purpose of Experiment 2 was to determine whether cognitive biases in approach–avoidant behavior to these stimulus cues are present in chicks subjected to a social-separation stressor known to induce either an anxiety-like state (Isolated-5 m) or a depression-like state (Isolated-60 m). As before, runway start latencies were unaffected by stimulus cues in the Non-isolated group. However, start latencies in the Isolated-5 m group were longer under stimulus cues containing greater amounts of aversive characteristics (i.e., 50c:50o, 25c:75o, and owl). Given that more pessimistic-like judgments are typically defined as enhanced avoidant behavior to ambiguous aversive stimuli, it follows that these chicks in an anxiety-like state show a cognitive bias best characterized as more pessimistic-like judgments. Moreover, start latencies in the Isolated-60 m group were not only longer under these same stimulus cue conditions, reflecting more pessimistic-like judgments, but also under the stimulus cues containing the greatest amounts of appetitive characteristics (i.e., chick and 75c:25o). Given that less optimistic-like judgments are typically defined as diminished approach behavior to ambiguous appetitive stimuli, it follows that these chicks in a depression-like state show a cognitive bias that includes not only more pessimistic-like, but also less optimistic-like judgments.

We anticipated finding a similar pattern of cognitive biases across the stimulus conditions using goal latency measures. However, goal latencies in the Isolated-5 m and Isolated-60 m groups were longer under only 50c:50o and owl stimulus cues (i.e., more pessimistic-like behavior) but not the intermediate cue of these two. Further, goal latencies in the Isolated-60 m group were longer in only the Chick cue (i.e., less optimistic-like behavior) but not the 75c:25o cue. The absence of a consistent finding across the two dependent measures of start and goal latency very likely reflects a ceiling effect imposed by the 5 m test session criterion. Indeed, a large number of chicks in the stress conditions did approach the stimulus cues to varying degrees but did not reach the goal box within the 5 m test session. Whether goal latency using a longer test session would reveal the same patterns of cognitive bias as start latency across experimental conditions is unknown. However, we believe the addition of runway distance traveled as a dependent measure may highlight cognitive biases to ambiguous appetitive and aversive cues in the chick anxiety–depression model that goal latency does not.

Observations of enhanced avoidant behavior to ambiguous aversive stimuli as well as diminished approach behavior to ambiguous appetitive stimuli in the current study are consistent with other findings in the literature. One study showed that European starlings exposed to standard cages after being exposed to enriched cages viewed ambiguous stimuli associated with food reinforcement more negatively (Bateson and Matheson, 2007). Furthermore, rats exposed to an unpredictable housing stressors (e.g., light:dark cycle, damp bedding) displayed reduced anticipation of a positive event, as indexed by fewer and slower responses to a tone, as well as ambiguous tones, previously paired with food reward (Harding et al., 2004). Expanding on the existing literature, we believe the current findings are the first to show 1) the runway test to ambiguous appetitive and aversive cues can assess both types of cognitive biases within a single stress model and 2) both types of cognitive biases are present in the depressive-like state within the chick anxiety–depression model. Moreover, it is interesting to note that the two forms of cognitive biases dissociate anxiety- and depressive-like states in a manner similar to earlier pharmacological dissociation studies (Sufka et al., 2006; Warnick et al., 2009).

Collectively, the observation that cognitive biases of both more pessimistic-like and less optimistic-like behavior present within the single test paradigm adds to the validity of the chick anxiety–depression model as a neuropsychiatric simulation. More importantly, it has been argued that both human and animal data suggest cognitive biases are critical elements in the pathogenesis of anxiety and depression and may elucidate common neurophysiological underpinnings of these stress related disorders (Kalueff and Murphy, 2007).

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 seen in these two seemingly related clinical disorders.

4. Experimental procedures

4.1. *Subjects*

Cockerels (*Gallus gallus*; W36; 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. In an attempt to minimize experimenter-related stress during later testing chicks were removed and briefly handled daily. Food (Purina Start and Grow, St Louis, Missouri, USA) and water were available ad libitum through one quart gravity-fed feeders (Murray MacMurray; Model 4BGFJ) and waterers (Murray MacMurray; Model 4YQW0). Room temperature was maintained at 29±1 °C and overhead illumination was maintained on a 12-h light–dark cycle from 0700 to 1900 h.

4.2. *Straight alley maze and morphed stimulus conditions*

The apparatus consists 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. The straight alley maze consists of a 10×10×10 cm start box with a guillotine door that opens up to a 40×10×10 cm runway with either an 8×10 cm mirror or various 8×10 cm stimulus cues placed at its end (detailed below). A 40×20×10 cm holding arena housed 12 conspecifics throughout the test session and permitted testing chicks under non-isolated treatment conditions. These conspecifics remained out of view during maze testing. However, once chicks reached the goal, full view of the arena was permitted through a 20×10 cm clear Plexiglas wall. Pine bedding was placed throughout the arena floor and food and water were available ad libitum in 200 ml stainless steel cups.

Morpheus Photo Morpher v3.01 Professional for Mac (Morpheus Software, LLC) was used to produce 'morphed' images that blended elements of a chick and a horned owl silhouette. From the chick and owl silhouette cues, a series of approximately 200 dots were selected from each photo and matched their location between the images. A software program then took these data points and produced 100 morphed frames linking the start (chick=c) and end (owl=o) photos. Within this series three 'key' frames were defined: one each at 75c:25o, 50c:50o, and 25c:75o ratios of chick to owl characteristics. The pixilated 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 detailed in Fig. 2 were saved as jpeg files, printed in graded color (yellow to black) and placed behind a clear glass plate during testing.

A pilot study demonstrated that 95% of non-isolated chicks exited the start box within 30 s (100% did exit the start box within the 5 m test session) and 92% reached the goal within 60 s of a 5 m test period under the mirror test condition. To determine whether the owl silhouette served as an aversive cue, a second pilot study was conducted using two test sessions separated by one day. The first test was conducted using the mirror cue and replicated the findings of the first pilot study. The second test utilized the owl silhouette cue and demonstrated that 88% of non-isolated chicks exited the start box within 30 s (100% did exit the start box within the 5 m test session) and 81% failed to reach the goal within the 5 m test period.

4.3. *Isolation apparatus*

A six-unit test apparatus containing Plexiglas viewing chambers (25×25×22 cm) situated within sound-attenuating enclosures was used for behavioral data collection. The units were illuminated using 25 W light bulbs and ventilated by an 8-cm diameter rotary fan (Model FP-108AXS1; Rodale, Great River, New York, USA). Miniature video cameras (Model PC60XP; SuperCircuit, Liberty Hill, Texas, USA) mounted at floor level in the corner of the enclosures and routed through a multiplexer (Model PC47MC; SuperCircuit) allowed for animal observation. Distress vocalizations were collected via microphones (Model 3-675-001 (modified); Lafayette Instruments, Lafayette, Indiana, USA) mounted on the rear wall of the Plexiglas chamber, routed through sound-activating relays (Model 630400A; Lafayette Instruments; settings: 40–75% sensitivity, 0.10-s delay) and collected in a USB interface via custom-designed software.

4.4. *Procedures*

For Experiment 1, chicks were tested twice at ages 4 and 5 days post hatch. In the first trial, 12 cagemate conspecifics were placed into the holding arena and individually tested in the maze under the mirror cue condition. Each chick was placed individually into the start box for 15 s after which the guillotine door was raised. Dependent measures were start and goal latencies. Start latency was defined as the time it took to step completely outside the start box. Goal latency was defined as the time to cross a defined mark located 10 cm away from the mirror or stimulus cue. A test session was terminated at 5 m if a chick had not reached the goal and a goal latency of 300 s recorded. The chick was placed back into the holding arena until all had been tested. Goal latencies from this test session were used as a basis for a balanced assignment to one of 5 stimulus cue conditions in the second trial. Test procedures and dependent measures for the second trial were identical to that of the first trial except that the mirror was replaced by one of the 5 stimulus cues.

In Experiment 2, chicks were tested across ages 4–6 days post hatch. At age 4, all chicks were tested under the mirror condition as described in Experiment 1. Goal latency data were used as a basis for a balanced assignment to one of the stimulus cue × isolation treatment conditions described below. The second trial was conducted at either 5 or 6 days post hatch. The isolation treatment conditions consisted of a non-isolated control group and two groups of isolated chicks. The non-isolated chicks were placed into the holding arena with testing performed as described in Experiment 1; these animals remained in the arena throughout testing of the isolated groups. Under the isolation stress condition, the chicks of one group were isolated for 5 m to induce the anxiety-like state prior to maze testing. The chicks of a second group were isolated for 60 m to induce the depression-like state prior to maze testing. Chicks were transported from the isolation apparatus in a 2-quart opaque plastic container and tested immediately in the maze. Dependent measures were as described in Experiment 1 but also included distress vocalization rates during the isolation manipulation. Chicks were returned to their home cage after testing. All procedures were approved by the University of Mississippi Institutional Animal Care and Use Committee (protocol 09-016).

REFERENCES

Bateson, M., Matheson, S.M., 2007. Performance on a categorization task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). Anim. Welf. 16, 33–36.

Belanoff, J.K., Rothschild, A.J., Cassidy, F., DeBattista, C., Baulieu, E.E., Schold, C., Schatzberg, A.F., 2002. An open label trial of C-1073 (mifepristone) for psychotic major depression. Biol. Psychiatry 52, 386–392.

Bethell, E.J., Semple, S., Holmes, M., MacLarnon, A., 2007. The effect of emotion state on responses to social stimuli by rhesus macaques. Primate Eye 92, 5–6.

Brilot, B., Normandale, C., Parkin, A., Bateson, M., 2009. Can we use starlings' aversion to eyespots as the basis for a novel 'cognitive bias' task? Appl. Anim. Behav. Sci. 118, 182–190.

Brilot, B.O., Asher, L., Bateson, M., 2010. Stereotyping starlings are more 'pessimistic'. Anim. Cogn. 13, 721–731.

Burman, O.H.P., Parker, R.M.A., Paul, E.S., Mendl, M., 2008. Sensitivity to reward loss as an indicator of animal emotion and welfare. Biol. Lett. 4, 330–333.

Clarke, C.H., Jones, R.B., 2001. Domestic chicks' runway responses to video images of conspecifics. Appl. Anim. Behav. Sci. 70, 285–295.

Cohen, L., de Moor, C., Amato, R.J., 2001. The association between treatment-specific optimism and depressive symptomatology in patients enrolled in a Phase 1 cancer clinical trial. Cancer 91, 1949–1955.

Eysenck, M.W., Mogg, K., May, J., Richards, A., Mathews, A., 1991. Bias in interpretation of ambiguous sentences related to threat in anxiety. J. Abnorm. Psychol. 2, 144–150.

Harding, E.J., Paul, E.S., Mendl, M., 2004. Animal behavior—cognitive bias and affective state. Nature 427, 312.

Jones, R.B., Marin, R.H., 1999. T-maze behavior, growth, sociality, and stress in unselected broiler chickens: strategic implications. Behav. Genet. 29, 360-360.

Kalueff, A.V., Murphy, D.L., 2007. The importance of cognitive phenotypes in experimental modeling of animal anxiety and depression. Neural Plast. 2007, 1–7.

Keppel, G., Wickens, T., 2004. Design and Analysis, fourth ed. Upper Saddle River, Prentice Hall.

Lehr, E., 1989. Distress call reactivation in isolated chicks: a behavioral indicator with high selectivity for antidepressants. Psychopharmacology (Berl) 97, 145–146.

MacLeod, A., Byrne, A., 1996. Anxiety, depression, and the anticipation of future positive and negative experiences. J. Abnorm. Psychol. 105, 286–289.

Marin, R.H., Jones, R.B., 2000. T-maze behavior in broiler chicks is not sensitive to right–left preferences, test order or time-of-day. Appl. Anim. Behav. Sci. 68, 207–214.

Marin, R., Freytes, P., Guzman, D., Jones, R., 2001. Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers. Appl. Anim. Behav. Sci. 71, 57–66.

Marin, R.H., Satterlee, D.G., Castille, S.A., Jones, R.B., 2003. Early T-maze behavior and broiler growth. Poult. Sci. 5, 742–748.

Matheson, S.M., Asher, L., Bateson, M., 2008. Larger enriched cages are associated with 'optimistic' response biases in captive European starlings (*Sturnus vulgaris*). Appl. Anim. Behav. Sci. 109, 374–383.

Mendl, M., Burman, O., Parker, R., Paul, E., 2009. Cognitive bias as an indicator of animal emotion and welfare: emerging evidence and underlying mechanisms. Appl. Anim. Behav. Sci. 118, 161–181.

Miczek, K., de Wit, H., 2008. Challenges for translational psychopharmacology research—some basic principles. Psychopharmacology 199, 291–301.

Miranda, R., Mennin, D.S., 2007. Depression, generalized anxiety disorder, and certainty in pessimistic predictions about the future. Cogn. Ther. Res. 31, 71–82.

Mogg, K., Baldwin, D.S., Brodrick, P., Bradley, B.P., 2004. Effect of short-term SSRI treatment on cognitive bias in generalized anxiety disorder. Psychopharmacology 176, 466–470.

Mogg, K., Bradbury, K.E., Bradley, B.P., 2006. Interpretation of ambiguous information in clinical depression. Behav. Res. Ther. 44, 1411–1419.

Panksepp, J., 2006. Emotional endophenotypes in evolutionary psychiatry. Prog. Neuropsychopharmacol. Biol. Psychiatry 30, 774–784.

Rowe, C., Skelhorn, J., 2005. Colour biases are a question of taste. Anim. Behav. 69, 587–594.

Schechter, L.E., Ring, R.H., Beyer, C.E., Hughes, Z.A., Khawaja, X., Malberg, J.E., Rosenzweig-Lipson, S., 2005. Innovative approaches for the development of antidepressant drugs: current and future strategies. Neurotherapeutics 25, 90–611.

Sufka, K.J., Feltenstein, M.W., Warnick, J.E., Acevedo, E.O., Webb, H. E., Cartwright, C.M., 2006. Modeling the anxiety–depression continuum hypothesis in domestic fowl chicks. Behav. Pharmacol. 17, 681–689.

Sufka, K.J., Warnick, J.E., Pulaski, C.N., Slauson, S.R., Kim, Y.B., Rimoldi, J.M., 2009. Antidepressant efficacy screening of novel targets in the chick anxiety–depression model. Behav. Pharmacol. 20, 146–154.

van der Staay, F.J., 2006. Animal models of behavioral dysfunctions: basic concepts and classifications, and an evaluation strategy. Brain Res. Rev. 52, 131–159.

Warnick, J.E., Wicks, R.T., Sufka, K.J., 2006. Modeling anxiety-like states: pharmacological characterization of the chick separation stress paradigm. Behav. Pharmacol. 17, 581–587.

Warnick, J.E., Huang, C.-J., Acevedo, E.O., Sufka, K.J., 2009. Modeling the anxiety–depression continuum in chicks. J. Psychopharmacol. 23, 143–156.

Willner, P., 1991. Behavioural models in psychopharmacology. In: Willner, P. (Ed.), Behavioral Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives. Cambridge University Press, Cambridge, pp. 3–18.

Wolkowitz, O.M., Reus, V.I., Keebler, A., Nelson, N., Friedland, M., Brizedine, L., Roberts, E., 1999. Double blind treatment of major depression with dehydroepiandrosterone. Am. J. Psychiatry 156, 646–649.

Wright, W.F., Bower, G.H., 1992. Mood effects on subjective probability assessment. Organ. Behav. Hum. Decis. Process. 52 (2), 276–291.

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006a. A randomized trial of an N-methyl-D-aspartate agonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63, 856–864.

Zarate, C.A., Singh, J., Quiroz, J.A., De Jesus, G., Lukenbaugh, D., Denicoff, K.D., Manji, H.K., Charney, D.S., 2006b. A double-blind, placebo-controlled study of memantine in the treatment of major depression. Am. J. Psychiatry 163, 153–155.