Modeling the anxiety–depression continuum hypothesis in domestic fowl chicks

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Anxiety and depression are currently classified as separate clinical syndromes despite considerable similarities in their symptoms, pathophysiological substrates and response to treatment interventions. An alternative hypothesis views anxiety and depression along a temporal continuum, a construct that the current research attempts to model in a preclinical setting. In experiment 1, socially raised domestic fowl chicks separated from conspecifics demonstrated a pattern of distress vocalizations that sequentially models anxiety-like and depressive-like states. In addition, administration of the benzodiazepine anxiolytic chlordiazepoxide and the tricyclic antidepressant imipramine provided pharmacological validation for the model in that they were capable of dissociating the anxiety-like and depressive-like states. In experiment 2, corticosterone levels were quantified across the isolation test session to provide convergent validity to the model. These findings fit well with the human clinical literature on the anxiety–depression continuum perspective, and suggest the consideration of a nosology that emphasizes the inter-relatedness of these clinical states rather than their boundaries. *Behavioural Pharmacology* **17:681–689**

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Introduction

The current *Diagnostic and statistical manual of mental disorders-IV* categorizes anxiety and depression as separate Axis I clinical syndromes (American Psychiatric Association, 1994). Such taxonomy, as originally outlined by Kraepelin's criteria, implies these syndromes have distinct etiological origins, symptom expression, pathophysiological substrates and treatment response outcomes. Evidence is, however, mounting to suggest anxiety and depressive disorders have more in common than previously thought. For example, anxiety and depressive disorders share many signs and symptoms (Watson, 2005) and present comorbidity rates ranging from 50 to 90% (Kessler *et al.*, 1994, 2005; Rivas-Vazquez *et al.*, 2004). Structural equation modeling suggests a close relationship between anxiety disorders and depression, with high zero order correlations and considerable contributions to each from the higher order factors of negative affect and low positive affect (Brown *et al.*, 1998). From an etiological perspective, three interacting vulnerabilities have been identified as important contributors to the expression of negative affect in anxiety and depressive disorders and include: (i) general biological vulnerabilities such as genetic contributions, (ii) general psychological vulnerabilities such as early life experiences of unpredictability and uncontrollability and (iii) specific psychological vulnerabilities such as faulty associative learning experiences (Barlow, 2000). While the underlying pathophysiological processes of anxiety and depression are diverse and complex, common biological markers exist and include dysregulation of glucocorticoids (Arborelius *et al.*, 1999), monoamines (Ressler and Nemeroff, 2000) and neurotrophic factors (Jiang *et al.*, 2005). Further, anxiety and depression share similar response rates to cognitive-behavioral therapy (Hollon *et al.*, 2006) and to certain classes of pharmacological agents, notably antidepressants (Feighner, 1999). Collectively, these findings bring into question whether genuine boundaries exist between these clinical disorders or are better served by a single overarching construct (Watson, 2005). For example, one model suggests that anxiety and depression may represent different temporal facets of a single syndrome in which the expression of the depressive state follows the anxiety state to an unresolved stressor (Kasper, 2001).

Considerable concerns are being raised over the validity of existing animal models of clinical syndromes (Frazer

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and Morilak, 2005; Matthews *et al.*, 2005; McArthur and Borsini, 2006; van der Staay, 2006). Much of this criticism seems to target the face and predictive validity of drug screening assays, in which the former concerns phenomenological similarities to clinical syndromes and the latter concerns correctly demonstrating efficacy of therapeutics on the behaviors of interest (Willner, 1986; Wright, 2002; McArthur and Borsini, 2006; but see also McKinney and Bunney, 1969). van der Staay (2006) argues that the most important validity measure of an animal model is construct validity, which refers to the theoretical underpinnings of the model. Construct validity is established by the use of sound experimental procedures and by empirical work developing a network of associations including homology to a clinical syndrome. Good construct validity, which certainly entails aspects of both face and predictive validity, includes quantification of measures or endophenotypes of a syndrome's symptomatology, noting constraints relative to an organism's own specific behavioral repertoire to a stressor (Sarter and Bruno, 2002), response to treatment and underlying neuropathology (Panksepp, 2006; van der Staay, 2006).

Indeed, understanding the neurophysiological substrates of and identifying novel treatment targets for human clinical syndromes are often dependent upon the development, validation and use of animal models (Willner, 1991a; van der Stay, 2006). Although numerous animal paradigms exist for modeling anxiety (Green and Hodges, 1991) and depression (Willner 1991b, 1997)-like syndromes, little attention has been given to simulating this hypothesized anxiety–depression continuum in a single paradigm. Development of such a model would not only add empirical support to a new taxonomy of mental disorders, but it may also shed light on the course, pathology and treatment of such a clinical syndrome.

Anxiety-like states have been modeled in domestic fowl chicks by recording distress vocalizations (DVocs) in response to brief social-separation stress (Panksepp *et al.*, 1978, 1980; Panksepp, 2003). Studies from this lab have shown the paradigm as an anxiety model to possess construct (Sufka and Weed, 1994; Feltenstein *et al.*, 2002), convergent (Feltenstein *et al.*, 2003a) and predictive validity (Watson and Sufka, 1996; Watson *et al.*, 1999; Feltenstein *et al.*, 2004; Feltenstein and Sufka, 2005; Warnick *et al.*, 2006) and we have utilized the model as an anxiolytic screening assay by demonstrating that a wide range of compounds reliably and dose-dependently reduce DVocs (Smith *et al.*, 2001; Sufka *et al.*, 2001; Feltenstein *et al.*, 2003b). Chick DVocs have also been used to model depression (Lehr, 1989; Panksepp *et al.*, 1991). In the Lehr (1989) study, chicks isolated for 2 h from conspecifics display a pronounced decline in rates of DVocs that appear to resemble a learned helplessness/'behavioral despair' response, a profile commonly associated with depression (Seligman *et al.*, 1968; Katz, 1981). Lehr (1989) further reported that the paradigm possesses predictive validity, in that a wide variety of antidepressant drugs reversed this state by enhancing total distress calls during the second hour of isolation whereas compounds lacking antidepressant activity did not. However, no time course data were provided in this study and it is possible that two clinical simulations may be conflated in the paradigm.

The present research sought to determine whether a 2-h chick isolation stress procedure sequentially models the two clinical states of anxiety and depression vis-à-vis the anxiety–depression continuum hypothesis. In experiment 1, DVocs were collected in 5 min blocks to characterize the temporal pattern of vocalizations displayed across the 2 h isolation test session. It was predicted that DVocs would present at their highest rate early in the test session, decline and then stabilize at a much lower rate in the latter half of the test session. Further, to dissociate putative anxiety-like and depression-like phases of the DVoc response, separate groups of isolated chicks were given either the benzodiazepine anxiolytic chlordiazepoxide or the tricyclic antidepressant imipramine. It was predicted that chlordiazepoxide would be effective in reducing DVocs only during the initial, anxiety-like phase but ineffective otherwise, whereas imipramine, which possesses both anxiolytic and antidepressant effects, would be capable of modulating both the early anxiety-like phase (i.e. reduction in DVocs) and the latter depressive-like phase (i.e. increase in DVocs) of the chick isolation stress response.

Corticosterone is a biological marker of stress in organisms and, as such, can serve as an important measure of convergent validity of the paradigm as a potent stress model. Thus, in experiment 2, we quantified plasma corticosterone levels at several intervals across the isolation session. We predicted that isolated chicks would show elevated corticosterone levels that would subsequently decline as a function of isolation duration owing to negative feedback processes of corticosterone on hypothalamic–pituitary–adrenal (HPA) axis activity.

Methods

Subjects

Cockerels (*Gallus gallus*, strain W-36; Cal-Maine Foods, Mendenhall, Mississippi, USA) were obtained 1 day after hatch and housed in stainless-steel cages ($44 \times 61 \times 40$ cm) at a population density of 12 chicks per cage. Food (Purina Start and Grow; Purina Mills, St Louis, Missouri, USA) and water were available *ad libitum* through a 1-quart gravity-fed feeders (Model 4BGFJ; Murray MacMurray, Webster City, Iowa, USA) and waterers (Model 4YQW0; Murray MacMurray). Room temperature was maintained at $29 \pm 1^{\circ}$C and overhead florescent

illumination was maintained on a 12-h light–dark cycle provided by fluorescent overhead lighting.

Apparatus

A six-unit test apparatus containing Plexiglas viewing chambers ($25 \times 25 \times 22$ cm) situated in 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. The microphones [Model 3-675-001 (modified); Lafayette Instruments, Lafayette, Indiana, USA] were mounted at the ceiling of the Plexiglas chamber and connected to digital sound-activating relays (Model 630400A; Lafayette Instruments; settings: 60–75% sensitivity, 0.10-s delay) that activated electromechanical counters (Model 58004; Lafayette Instruments). A white noise generator (Model 15800; Lafayette Instruments) provided masking noise in the test room.

Procedure

In experiment 1, tests were conducted during the middle three-quarters of their light cycle across a 3-day period, at ages 4–6 after hatch. The four experimental groups for this study included vehicle-treated chicks tested with two noncage mate conspecifics, vehicle-treated chicks tested in isolation, chlordiazepoxide-treated chicks tested in isolation and imipramine-treated chicks tested in isolation ($n = 12$); the latter two groups were included to dissociate putative anxiety-like and depressive-like states. The vehicle was 0.9% physiological saline and the doses of the probes were 8 mg/kg chlordiazepoxide (Research Biochemicals, Inc., Natick, Massachusetts, USA) and 15 mg/kg imipramine (Sigma-Aldrich, St Louis, Missouri, USA); these doses were selected from our previous work showing their ability to reduce DVocs significantly (approximately an ED_{50}) during a 3-min isolation period. Vehicle and drug injection were administered intraperitoneally 15 min before tests and the observation session was a 120-min test period. The dependent measure of DVocs was collected in 5 min time blocks. Animals were tested only once and returned to their home cage following these experimental procedures. These protocols were approved by the University of Mississippi's Institutional Animal Care and Use Committee (protocol no. 04-020).

In experiment 2, tests were conducted at 4 or 5 days after hatch during the middle half of their light cycle. The experimental design consisted of seven treatment conditions that included one social group (test length = 60 min) and six isolated groups (test lengths = 5, 10, 15, 20, 40 and 60 min). Sample sizes for the behavioral measure were $n = 12$. Following behavioral data collection, half of the animals from each test squad were taken from the test apparatus and rapidly decapitated for blood collection. To determine basal corticosterone levels, blood samples were obtained in a group of chicks killed upon removal from their home cage before conducting behavioral tests. All blood samples ($n = 6$) were collected in ethylenediaminetetraacetic acid tubes and were immediately chilled. Blood samples were centrifuged for 15 min at 3000 r.p.m. Supernatants were collected and stored at -80°C until analysis. Plasma corticosterone levels were determined using radioimmunoassay (Coat-a-Count RIA Kit; Diagnostic Products, Los Angeles, California, USA). The radioimmunoassay inter-assay and intra-assay coefficient of variations were 2.72 and 1.84%, respectively and the R^2 for the standards was 0.982. These protocols were approved by the University of Mississippi's Institutional Animal Care and Use Committee (protocol no. 05-008).

Data analyses

Data were analyzed using one-way and two-way repeated-measures analysis of variance (ANOVA) and one-way simple effects ANOVA. Post-hoc analyses were conducted using Fisher's least significant difference tests. Regression analyses were performed to test for quadratic function in the corticosterone time course response.

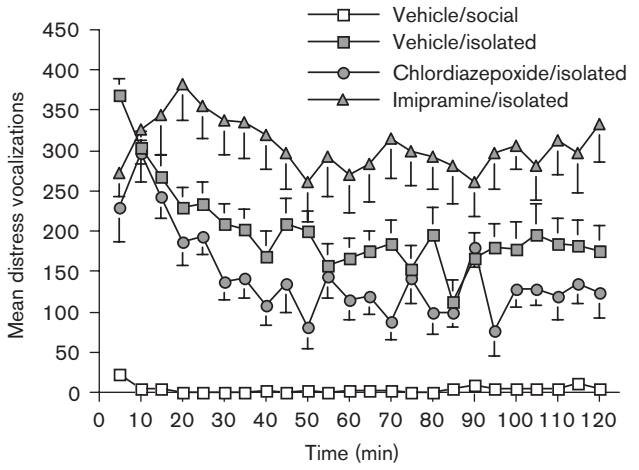
Results

Experiment 1: temporal distress vocalization response and chlordiazepoxide and imipramine effects

The effects of the two drug probes on separation-induced DVocs are summarized in Fig. 1. Vehicle chicks tested in the presence of two social companions exhibited few, if any, DVocs during the course of the test session. Isolation from social companions produced a robust DVoc response in vehicle chicks. The rate of DVocs declined over the first 30 min of the test session to approximately 50% of the initial response rate and remained relatively stable thereafter. At the 5 min block, both chlordiazepoxide and imipramine attenuated DVocs. At the 20 min block and beyond, however, where vocalization rates had declined, imipramine enhanced DVoc rates. During this same time period, chlordiazepoxide did not generally alter DVoc rates from that of isolated chicks.

Consistent with these descriptions, a two-way ANOVA revealed significant main effects for Treatment [$F(3,1012) = 33.44$, $P < 0.001$], Time [$F(23,1012) = 7.67$, $P < 0.001$] and a significant Treatment × Time interaction [$F(69,1012) = 2.59$, $P < 0.001$]. A one-way repeated-measures analysis for the vehicle-social group revealed a significant effect for Time [$F(23,253) = 3.46$, $P < 0.001$]. Post-hoc analyses demonstrated that the only consistent and relevant difference in DVoc rates across time blocks

Fig. 1



Behavioral characterization and pharmacological validation of the chick anxiety–depression continuum model. Data points represent means ± SEM ($n = 12$). Compared with chicks tested in the social condition (open symbol), isolated chicks (filled symbols) displayed a significant increase in distress vocalizations (DVocs) that was maximal during the first 5 min block (anxiety-like state), significantly declined over the next 20 min (transitional phase) and stabilized at approximately 50% the initial rate for the remainder of the session (depressive-like state). The benzodiazepine anxiolytic chlordiazepoxide significantly attenuated DVocs during the anxiety-like phase but did not generally affect DVoc rates thereafter. The tricyclic antidepressant imipramine significantly decreased DVocs during the protest phase and significantly increased DVocs during the depression-like phase (Ps < 0.05).

was that chicks vocalized significantly more in the first 5 min than each of the remaining test session blocks (Ps < 0.005). To examine the pattern of DVocs in isolated chicks, a one-way repeated measures for the vehicle-isolated group was performed and revealed a significant effect for Time [$F(23,253) = 7.36$, $P < 0.001$]. The results of post-hoc analyses highlight three noteworthy patterns. First, DVocs were highest at the 5 min time block and significantly lower for each of the remaining test session blocks (Ps < 0.05). Second, there were no differences in DVoc rates between the 10 and 15 min time blocks or the 15 and 20 min time blocks as DVoc rates gradually declined; DVoc rates for the 30–120 portion of the test session were significantly lower than the 5–15 min period (Ps < 0.05). Finally, DVoc rates were generally stable across the 20–120 min portion of the test session (Ps < 0.05).

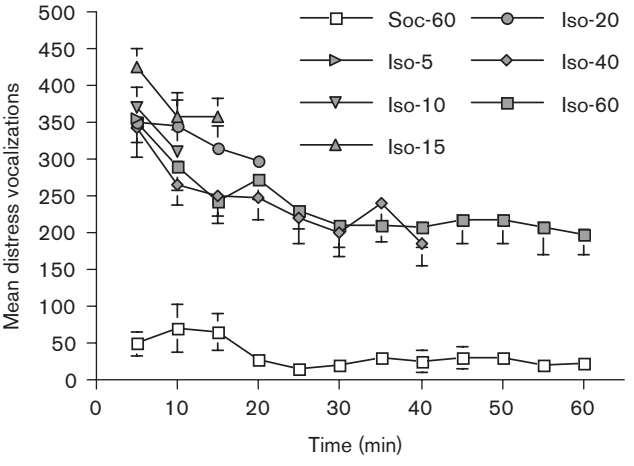
To examine the effects of the drug probes on patterns of DVocs, one-way ANOVAs were conducted at each test block. A one-way ANOVA at the first 5 min time block revealed a significant Treatment effect [$F(3,44) = 24.53$, $P < 0.001$]. Post-hoc analyses demonstrated that the vehicle-isolated group vocalized significantly more than the vehicle-social group and that imipramine and chlordiazepoxide significantly reduced DVocs in isolated groups (Ps < 0.001). One-way ANOVAs at the 10 min [$F(3,44) = 25.40$, $P < 0.001$] and 15 min [$F(3,44) = 21.50$, $P < 0.001$] time blocks revealed significant Treatment effects. In both sets of analyses, post-hoc tests demonstrated that DVocs were significantly higher in all three isolated groups than in the vehicle-social group (Ps < 0.001); no other relevant comparisons (i.e. effects of drug probes) reached statistical significance.

A one-way ANOVA at the 20 min time block revealed a significant Treatment effect [$F(3,44) = 27.24$, $P < 0.001$]. Post-hoc analyses demonstrated that while vehicle-isolated DVoc rates had declined from their initial rate, they were still significantly higher than vehicle-social chicks ($P < 0.001$). Interestingly, the decline in DVoc rates in vehicle-isolated chicks was reversed in chicks receiving imipramine ($P < 0.001$); chlordiazepoxide did not affect DVoc rates at this time block. In general, this pattern of treatment effects on DVoc rates remained consistent throughout the remainder of the test session [e.g. at 60 min, $F(3,44) = 13.46$, $P < 0.001$ and at 120 min, $F(3,44) = 17.97$, $P < 0.001$].

Experiment 2: temporal distress vocalizations and corticosterone responses

The effects of varying lengths of social separation on DVocs from experiment 2 are summarized in Fig. 2. In animals tested in the social condition, DVocs were

Fig. 2



Mean (± SEM) distress vocalization (DVocs) in the social (Soc) (open symbol) and isolated (Iso) (filled symbols) test conditions as a function of test session length ($n = 12$). Chicks tested in the social condition displayed low and stable rates of DVocs over the 60 min test session. Chicks in each isolation test condition displayed a significant increase in DVoc rates. The pattern of vocalizations is as described in Fig. 1 in which DVoc rates are highest during the first 5 min block marking the anxiety-like state and significantly declined within 20–25 min to approximately 50% the initial response rate and remained relatively stable throughout the test session marking the depressive-like state (Ps < 0.05).

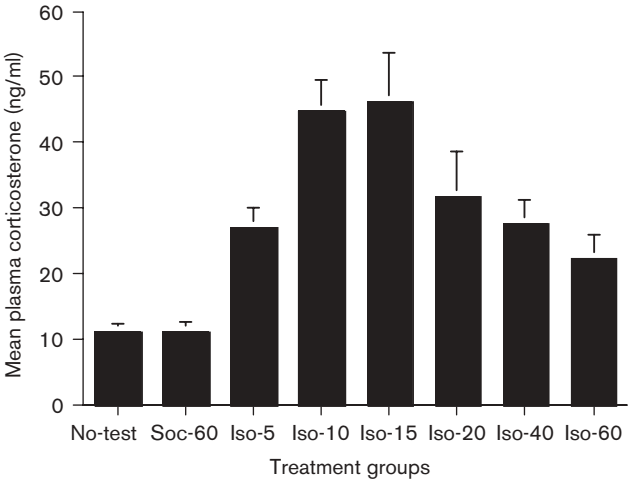
infrequent and remained stable across the 60 min test session. For all of the other test groups, isolation from conspecifics produced a robust elevation in DVocs. This increase in DVocs gradually declined over the initial 20 min period by approximately 30–40%, marking the emergence of the depression-like phase. Thereafter, DVocs remained relatively stable throughout the remainder of the test session.

A time course analysis of the vocalization data in the social-60 min and isolated-60 min groups illustrates both the stress effect of social separation and the emergence of learned helplessness in isolated chicks. Thus, a two-way repeated-measures ANOVA conducted for these two groups revealed a significant main effect for Treatment [$F(1,220) = 78.51$, $P < 0.001$], a significant main effect for Time [$F(11,220) = 5.15$, $P < 0.001$] and a significant Treatment × Time interaction [$F(11,220) = 2.13$, $P < 0.05$].

A one-way repeated measure for the vehicle-social group revealed a significant effect for Time [$F(11,110) = 1.91$, $P < 0.05$]. Post-hoc analyses demonstrated that the only consistent and relevant difference in DVoc rates across time blocks was that chicks vocalized significantly more at the 10 and 15 min blocks than each of the other test session blocks (Ps < 0.05). To examine the development of behavioral despair/learned helplessness, a one-way repeated measure for the vehicle-isolated group was performed and revealed a significant effect for Time [$F(11,110) = 4.29$, $P < 0.001$]. The results of post-hoc analyses highlight three noteworthy patterns. First, DVocs were highest at the 5 min time block and significantly lower for each of the remaining test session blocks (Ps < 0.05). Second, there was no difference in DVoc between the 10, 15 and 20 min time blocks as DVoc rates gradually declined. Finally, DVoc rates were stable across the 25–60 min portion of the test session and these rates were significantly lower than the 5 and 10 min blocks (Ps < 0.05).

The effects of varying lengths of social separation stress on plasma corticosterone are summarized in Fig. 3. Levels of corticosterone in the social-60 min group were comparable to the no-test control group. Levels of corticosterone in the isolated groups were elevated and marked by a peak in the isolated-10 min and isolated-15 min groups, and a gradual decline in the 20, 40 and 60 min isolation conditions. To examine the effects of exposure to the testing protocol and the effects of social separation on plasma corticosterone levels, a one-way ANOVA was conducted on these data in the no-test, social-60 min and isolation-60 min groups. This analysis revealed a significant effect for Treatment [$F(2,14) = 8.88$, $P < 0.005$]. Post-hoc analyses of these data revealed that mean plasma corticosterone levels were significantly higher for the isolated-60 min condition than the no-test and social-60 min groups ($P < 0.005$). To determine the effects of varying lengths of isolation on plasma corticosterone, a one-way ANOVA was conducted on data across the isolated groups. This analyses revealed a significant main effect for Time [$F(5,29) = 3.512$, $P < 0.05$]. Post-hoc analyses demonstrated that mean plasma corticosterone levels were significantly higher for the 10 and 15 min groups than for the 5, 40, and 60 min groups (Ps < 0.05). This inverted U-shaped function was confirmed by regression analyses that revealed a significant quadratic function in the relationship between isolation length and corticosterone levels in isolated chicks (Ps < 0.05).

Fig. 3



Mean plasma corticosterone levels (ng/ml) for no-test, socially tested (Soc), and isolated (Iso) groups ($n = 6$). Corticosterone levels were significantly higher for all the isolation conditions than the no-test and social-60 min groups. Among the isolated groups, corticosterone levels were the highest in the 10 and 15 min groups than the 5, 40, and 60 min groups. This inverted U-shaped function was confirmed by regression analyses that revealed a significant quadratic function in the relationship between distress vocalization rate and corticosterone levels in isolated chicks (Ps < 0.05).

Discussion

Empirical findings in the chick anxiety–depression continuum model

Separation from conspecifics in chicks elicits high rates of DVoc that decline over the course of the 2 h isolation experience. Both Lehr (1989) and Panksepp *et al.* (1991) describe the initially higher rate of DVocs as a 'protest' phase and the subsequent lower rate as either a 'resignation' (Lehr, 1989) or 'despair' (Panksepp *et al.*, 1991) phase. When looking more closely at the pattern of DVoc rates over time and the differential effects of chlordiazepoxide and imipramine on DVoc rates, however, we suggest that this stress response may be better characterized as three distinct phases we describe as

(i) an anxiety-like state, (ii) a transitional phase and (iii) a depressive-like state.

Separation-induced DVocs in chicks are aimed at re-establishing social contact (Gallup and Suarez, 1980) and are at their highest rate during the first 5 min block of the isolation test session. We are uncomfortable using 'protest' to describe DVoc behavior as this term suggests a 'strong disapproval' of social separation; rather, this appetitively motivated behavioral response is more akin to a panic or fear state elicited by the sudden onset of a stressor. Evidence that this initial phase models an anxiety-like state is provided by the observation that both chlordiazepoxide and imipramine, each of which possesses potent anxiolytic properties in humans (Baldessarini, 2001), attenuate DVocs during this time block. Furthermore, these findings are consistent with our previous work demonstrating that a wide range of anxiolytics and antidepressants possess anxiolytic properties in chicks briefly isolated (3 min) from social companions (Feltenstein *et al.*, 2004; Feltenstein and Sufka, 2005). Interestingly, recent research from this lab (Warnick *et al.*, 2006) has shown that anxiolytics clinically effective for panic disorder but not for generalized anxiety disorder attenuate DVocs during a 3-min social-separation stress period and suggest that DVocs in the initial anxiety-like phase described in the current study may model a more specific anxiety state in chicks.

During the next 10–15 min, DVoc rates display a steady decline. During this transitional phase, both chlordiazepoxide and imipramine lose their anxiolytic activity and imipramine begins to show evidence of its antidepressant activity. We believe that this phase marks the period anxiety-like states transition to depressive-like states and likely involves numerous dynamic neurochemical processes in limbic emotive and brainstem motivational structures. The underlying neurochemical processes in humans exposed to persistent stressors and experiencing anxiety and/or depressive symptoms include increases in glucocorticoids (e.g. cortisol, corticotropin releasing factor and adrenocorticotropic hormone), increased cytokinin productions (e.g. interleukin-6 and interleukin-1β), decreased neurotrophic factors (e.g. brain-derived neurotrophic factor), and depletion and decreased release of monoamines (e.g. norepinephrine and serotonin; for reviews see Tafet and Bernardini, 2003; O'Brien *et al.*, 2004). Research focused on this phase in the chick model may provide a better understanding of the neurochemical bases of and relationships between anxiety and depression and may reveal targets for novel treatment strategies.

We describe the final phase of the stress response as the depressive-like state and it is characterized by a reduced (40–50% of the initial rate) and stable pattern of DVocs throughout the remainder of the test session. Lehr (1989) and Panksepp *et al.* (1991) have described this reduced rate of DVocs as either 'resignation' or 'despair' and argue effectively that it models a depressive-like syndrome. It is interesting to consider whether this model better reflects behavioral despair or learned helplessness. In behavioral despair models like the Porsolt forced swim test (Porsolt *et al.*, 1977), animals become immobile after an initial escape attempt. In contrast, chick DVocs do not cease altogether but remain stable at a greatly reduced rate of expression. Learned helplessness models involve exposure to uncontrollable stressors and performance deficits on subsequent learning tasks (Seligman *et al.*, 1968). In addition to learning deficits, learned helplessness models also show a variety of other disturbances including poor performance in appetitively motivated tasks such as intracranial self-stimulation (Willner, 1991b). Given the function separation-induced DVocs have in chicks and the decline in their rates during a persistent stressor, one could argue that this paradigm has features that exist in learned helplessness models. Further evidence that this third phase models a depressive-like state is provided by both Lehr (1989) and Panksepp *et al.* (1991) in that a wide variety of antidepressants effectively reverse the reduction in DVoc rates (i.e. increase DVocs). Our data also show that imipramine, but not chlordiazepoxide, displays antidepressant activity in the model but, interestingly, does so only in the third phase which highlights the importance of a more fine-grained temporal analyses of chick vocalizations in the model. One other notable finding is that like 'behavioral despair' models, the chick isolation paradigm shows sensitivity to acute drug administration procedures. The effectiveness of acute drug administration procedures in learned helplessness models is equivocal (Willner, 1991b). It appears the chick anxiety–depression continuum model may possess certain features that dovetail into many kinds of depression models.

One additional interesting finding from experiment 1 is that chlordiazepoxide, while attenuating the anxiety-like phase, did not prevent the onset of the depressive phase and that imipramine, while attenuating the depressive phase, did so only by returning DVocs to their higher panic-like rates rather than reducing them to levels displayed by nonisolated chicks. We hypothesize that this may be due to the persistent nature of the stressor (i.e. continued isolation) and the temporal pattern of the neurochemical cascade that act on distinct systems that subserve the two behavioral states. This implies that anxiety and depression, while being temporally related, do present as separable facets of a single disorder. Rather than viewing the inability of drug probes to either prevent or completely reverse different phases of the model as a weakness, it can be viewed as one of its strengths. That is, the model not only defines and distinguishes pharmacological activity of different classes

of drug compounds, but it can also differentiate multiple clinical actions of a single drug compound. For a screening assay, the ability to screen simultaneously for efficacy in two clinical activities greatly enhances model utility.

The second experiment sought to further validate the anxiety–depression continuum model by quantifying the temporal pattern of corticosterone response in chicks subjected to varying lengths of isolation stress. As no appreciable changes were detected in DVoc rates during the latter half of the 2 h test session in experiment 1, the length of the test session in experiment 2 was shortened to 60 min. As in the first experiment, chicks tested in the social condition displayed relatively few DVocs (< 50/block) across the test session (see Fig. 2). In the 60 min isolation condition, DVocs averaged approximately 350 in the first 5 min block, gradually declined over the next 10–15 min, and then stabilized between 200 and 250/block throughout the remaining portion of the testing session. All other isolated groups followed this temporal pattern that is consistent with the three phases of the isolation stress response outlined earlier.

Corticosterone is a biological marker of stress in organisms and is released by the adrenal glands in response to increased hypothalamic–pituitary activity. In the present study, plasma corticosterone levels were comparable in the social-60 min and no-test conditions (i.e. 11.29 and 11.13 ng/ml, respectively; see Fig. 3). One could argue that chicks tested in the social-60 min condition might have shown a modest and transient increase in corticosterone levels over the course of the test session owing to exposure to the experimental procedures. Indeed, previous work from this lab has shown that 15 min of isolation in chicks tested with mirrors (to simulate a social condition) show an elevated corticosterone response that was approximately 50% of that found in isolated chicks tested under a no-mirror condition (Feltenstein *et al.*, 2003a). Whether a similar pattern occurs in chicks isolated with social companions was not addressed in this study.

Across all isolated groups, corticosterone was significantly elevated (22–46 ng/ml). Peak corticosterone levels were observed in the isolated-10 min and isolated-15 min chicks. Thereafter, levels declined over the remainder of the test session. This inverted U-shaped pattern of release is consistent with research demonstrating that (a) social separation is a potent stressor in chicks (Feltenstein *et al.*, 2003a) and (b) that corticosterone produces negative feedback on HPA axis functioning in response to repeated exposure to a stressor (Jaferi *et al.*, 2003; Jaferi and Bhatnagar, 2006). In the present study, the social-separation stressor is continuously present and research suggests that emotional stressors tend to produce rapid negative feedback on HPA axis activity (Sapolsky *et al.*, 1990; Plotsky *et al.*, 1993).

It is interesting to note the parallels between the present findings and the clinical research on anxiety and depressive disorders. An emerging body of literature implicates subtle but heightened HPA axis activity in a number of anxiety disorders (Reul and Holsboer, 2002; Risbrough and Stein, 2006) including panic disorder (Abelson and Curtis, 1996; Young *et al.*, 2004). On the other hand, the literature on HPA axis dysfunction in depressive disorders is extensive (Nemeroff and Vale, 2005; Schiepers *et al.*, 2005; Bale, 2006). Although short-term physiological stress responses likely promote organism homeostasis and survival, chronic exposure to stressors leads to HPA axis dysfunction and maladaptive physiological responses. Indeed, Hennessy *et al.* (2004) argue that the behavioral sequelae to an acute stressor of maternal separation in guinea pig pups resembles that following an infection, and the two may share common physiological substrates. Such behavioral changes of decreased exploratory activity/social interactions and increased piloerection and shivering that lead to hyperthermia following a global immune reaction are adaptive and promote recovery. This notion may very likely generalize to this chick model in that brief (5–15 min) social separation stress produces hyperthermia (Sufka and Hughes, 1991; Sufka and Weed, 1994). Although not measured in the current study, additional behavioral and physiological measures, such as general activity, body temperature and cytokine levels, during the 2 h isolation-stress paradigm would certainly enhance the construct validity of the chick isolation paradigm modeling an anxiety–depression continuum.

Validity and utility of the chick anxiety–depression continuum model

Animal models of clinical syndromes are used to answer a wide range of research questions from characterizing a syndrome's etiology and underlying pathophysiology to testing efficacy of novel therapeutics. The kinds of criteria to establish the validity of an animal model are dependent on whether that model is intended to serve as a simulation or a drug screening assay (Willner, 1991a, b; van der Staay, 2006). As a simulation, the chick anxiety–depression continuum model appears to possess face, construct and predictive validity in that (i) the procedure involves a potent identifiable stressor, (ii) the pattern of DVocs sequentially models a anxiety-like state followed by a depressive-like state and (iii) the dependent measure shows the appropriate sensitivity to an anxiolytic and an antidepressant probe. Adding convergent validity to the model is the demonstration that corticosterone release parallels that produced by emotional stressors. Several limitations of the model as a simulation, however, do exist and can be addressed by additional studies incorporating the addition of a chronic mild stressor and

chronic drug administration procedures to the model. Nevertheless, these initial findings provide empirical support of the chick anxiety–depression continuum model as a simulation and fit well with human clinical data outlined earlier that argues adopting a new taxonomic structure that highlights the inter-relatedness of these clinical syndromes rather than their distinct boundaries. Whether a major restructuring of these clinical categories occurs in the *Diagnostic and statistical manual of mental disorders-V*, intended for release in 2010, is unknown at this time.

Separation-DVocs (i.e. social or maternal) in young animals have been used in numerous species as an endophenotype of emotional disorders and it has been argued that such stress paradigms with clear functional homology display strong face and predictive validity (Olivier *et al.*, 1994; Miczek *et al.*, 1995; Rodgers, 1997). Much of the earlier work in domestic fowl leading to this study (Lehr, 1989; Panksepp *et al.*, 1991; Watson and Sufka, 1996; Watson *et al.*, 1999; Smith *et al.*, 2001; Sufka *et al.*, 2001; Feltenstein *et al.*, 2003b, 2004; Feltenstein and Sufka, 2005; Warnick *et al.*, 2006) already favors the chick anxiety–depression continuum model as a valid screening assay in that it is sensitive to a wide range of classes of anxiolytics and antidepressants, and insensitive to false positives (i.e. a compound shows efficacy in the model but later fails in clinical trails) and false negatives (i.e. a compound fails to show efficacy in the model but is effective in clinical populations).

How this chick paradigm functions as a dual pharmacological screen is as follows: isolation-induced DVocs are maximal at the first 5 min of the test session and marks the anxiety-like phase. Anxiolytic drug activity would be assessed during this period. A transitional phase occurs during the next 10–15 min of social isolation. The final phase begins at about the 20 min block and marks the beginning of the depression-like phase. This is characterized by reduced and stable DVocs for the remainder of the test session in which antidepressant drug activity would be assessed. It may be unnecessary to subject animals to the entire 1–2 h test session to effectively model depression and screen for drug efficacy. In fact, antidepressant effects of imipramine were detected as early as the 20 min time block and in as little as a single 5 min block. While longer test sessions (e.g. up to 40 or 60 min) do effectively capture the depression component and likely reduce variability, it would be at the expense of model utility.

The advantages of this chick anxiety–depression continuum model as a pharmacological screening assay over rodent-based anxiety or depression models are numerous. Using the criteria outlined by Willner (1991a, b), this assay possesses high utility and high-throughput for an in-vivo screening as it (i) uses a low-cost animal ($0.50 a chick), (ii) tests at a young age, (iii) uses a single relatively short test session (< 1 h), (iv) measures a species-typical response that is easily recorded, (v) screens for two drug properties in a single test and (vi) requires simple statistical analyses. Furthermore, this chick model appears to address the National Institutes of Health's 3R policy to Reduce, Refine and Replace animals in research (Russell and Burch, 1959). The model reduces the number of purpose-bred research animals as male chicks are a by-product of the commercial egg-laying industry and discarded at hatch. The model possesses a refined methodology as it minimizes the stress-provoking stimuli to a single, relatively short (20–30 min) test session. And finally, the model replaces the standard rodent-based models of anxiety and depression with a phylogenetically lower and, perhaps, less sentient species. While rodent models will likely continue to be the mainstay in the biomedical research, the attributes outlined above strongly argue for adopting the chick anxiety–depression continuum model as a supplement to rodent models as an early preclinical dual anxiolytic/antidepressant screening assay.

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