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Modelling the anxiety-depression continuum in chicks

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Abstract

The clinical syndromes of anxiety and depression are now thought to exist along a temporal continuum and this construct has been modelled in a preclinical setting in chicks separated from conspecifics. This research sought to further the validity of the chick anxiety-depression continuum model. Dose–response studies using two classes of anxiolytics (chlordiazepoxide: 2.5, 5.0, 10.0, 15.0 mg/kg, and clonidine: 0.1, 0.15, 0.2, 0.25 mg/kg) and three classes of antidepressants (imipramine: 1.0, 3.0, 10.0, 15.0 mg/kg, maprotoline: 2.5, 5.0, 10.0, 20.0 mg/kg and fluoxetine: 1.0, 5.0, 10.0, 20.0 mg/kg) showed an ability to detect anxiolytic activity of chlordiazepoxide, clonidine, imipramine and maprotoline in the anxiety-like phase of the model and to detect antidepressant effects of imipramine, maprotoline and fluoxetine in the depression-like phase of the model. In addition, blood plasma interleukin-6, a biomarker of stress, was found to be elevated in response to social-separation stress. Collectively, these findings further characterize the model as a simulation of the anxiety-depression continuum and begin to establish the paradigm as a high-utility adjuvant to rodent screening assays for putative anxiolytic and antidepressant compounds.

Key words

animal model; anxiety; depression; domestic fowl; separation stress

Introduction

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and the International Statistical Classification of Diseases and Related Health Problems (ICD-10) classify anxiety and depression as separate clinical syndromes (American Psychiatric Association, 2000; World Health Organization, 2004). Recent evidence suggests, however, that these two disorders have much in common. For example, anxiety and depressive disorders share many signs and symptoms (Watson, 2005) and present co-morbidity rates ranging from 50% to 90% (Kessler, *et al*., 2005; Kessler, *et al*., 1994; Rivas-Vazquez, *et al*., 2004). From an aetiological perspective, three interacting vulnerabilities have been identified as significant contributors to the expression of negative affect in anxiety and depressive disorders (Brown, *et al*., 1998) and include 1) genetic contributions, 2) early life experiences of unpredictability and uncontrollability and 3) faulty associative learning experiences (Barlow, 2000).

Although 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), neurotrophic factors (Jiang, *et al*., 2005) and gamma-aminobutyric acid (Kalueff and Nutt, 2007). Furthermore, anxiety and depression share similar response rates to cognitive-behavioural therapy (Hollon, *et al*., 2006) and to certain classes of pharmacological agents, notably antidepressants (Feighner, 1999). Collectively, these observations suggest that anxiety and depressive disorders may be better served by a single overarching construct (Watson, 2005). One perspective, the anxiety-depression continuum theory, suggests that these syndromes represent different temporal facets to persistent and unresolved stressors, where anxiety-like symptoms are a prelude to depressive-like symptoms (Baldwin, *et al*., 2002; Boyer, 2000; Kasper, 2001; Liebowitz, *et al*., 1990; Merikangas, *et al*., 2003; Paul, 1988; Polani, 2004; Tafet and Smolovich, 2004).

Identification of novel treatment targets for human clinical syndromes often depend upon the development, validation and use of animal models (van der Staay, 2006; Willner, 1990a). However, many concerns over the validity of animal models have been raised in recent years (Frazer and Morilak, 2005; Matthews, *et al*., 2005; McArthur and Borsini, 2006; van der Staay, 2006), particularly the predictive validity of drug screening assays, which are primarily concerned with correctly

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showing the efficacy of therapeutics on the behaviours of interest (McArthur and Borsini, 2006; Willner, 1986; Wright, 2002; but see alsoMcKinney and Bunney, 1969). Van der Staay (2006) argues that any clinical simulation should aim for good construct validity, which refers to the theoretical underpinnings of the model. Good construct validity, which entails good predictive validity, includes quantification of a syndrome's symptomatology, noting constraints relative to an organism's own specific behavioural repertoire to a stressor (Sartor and Bruno, 2002) and response to treatment and underlying neuropathology (Panksepp, 2006; van der Staay, 2006). With evidence accruing that anxiety and depression may reflect a single syndrome, the development and validation of such a simulation may shed light on the course, pathology and treatment of such a clinical disorder (Anisman and Matheson, 2005; Kalueff and Tuohimaa, 2004; Kalueff, *et al*., 2007).

Although many paradigms exist that model anxiety- (Carobrez and Bertoglio, 2005; Green and Hodges, 1990; Lang and Davis, 2006; Millan and Brocco, 2003) and depression- (e.g., Chourbaji, *et al*., 2005; Cryan, *et al*., 2005; Willner, 1990b; 1997) like syndromes in rodents, few seem capable of being integrated in such a way to model an anxiety-depression continuum. However, anxiety-like states have been modelled in domestic fowl chicks by recording distress vocalizations (DVocs) in response to brief social-separation stress (Panksepp, 2003; Panksepp, *et al*., 1980; Panksepp, *et al*., 1978). Studies have shown this anxiety model to possess construct (Feltenstein, *et al*., 2002; Feltenstein, *et al*., 2003; Sufka and Weed, 1994; Warnick, *et al*., 2006) and predictive validity (Feltenstein and Sufka, 2005; Feltenstein, *et al*., 2004; Warnick, *et al*., 2006; Watson, *et al*., 1999; Watson and Sufka, 1996). In particular, we have shown that this model is sensitive to drug probes that are used clinically in the treatment of panic disorder (i.e., phenelzine, imipramine, alprazolam and clonidine) but not generalized anxiety disorder (i.e., buspirone and trazodone) and have argued the simulation most closely resembles situationally bound panic disorder because the symptom onset is rapid, intense and brief with clear aetiological origins (Warnick, *et al*., 2006). Furthermore, chick social-separation-DVocs have also been used to model depression (Lehr, 1989; Panksepp, *et al*., 1991). In a study conducted by Lehr (1989), chicks isolated for 2 h from conspecifics displayed a pronounced decrease in DVoc rates that appeared to resemble a learned helplessness/behavioural despair response, a profile commonly associated with depression (Katz, 1981; Seligman, *et al*., 1968). Lehr (1989) further reported that this 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. Unfortunately, no time course data were provided that might have showed the presence of an anxiety-like state preceding the learned helplessness phase.

Recent research in this laboratory incorporated features of the aforementioned assays in an attempt to model an anxiety-depression continuum in chicks (Sufka, *et al*., 2006). In this study, separate groups of 4 to 6-day-old chicks received saline, 8 mg/kg chlordiazepoxide or 15 mg/kg imipramine and then placed into isolation chambers for a 2-h test session, where DVocs were recorded in 5-min blocks. Social-separation elicited high-DVoc rates that decreased over the course of the 2-h isolation experience. During the first 5-min block, DVoc rates were at their highest. This appetitively motivated behavioural response to re-establish social contact, much like a panic response, was elicited by the sudden onset of the social-separation stressor. Evidence that this initial phase modelled an anxiety-like state was provided by the observation that both chlordiazepoxide and imipramine, each of which possesses anxiolytic properties in humans (Baldwin, *et al*., 2002), decreased DVocs during this time block. During the next 10–15 min, DVoc rates displayed a steady decrease as a learned helplessness/behavioural despair state emerged. During this transitional period, both chlordiazepoxide and imipramine lost their anxiolytic activity, and imipramine began to show evidence of its antidepressant activity. The final phase of the stress response was the depressive-like state and it was characterized by a reduced (40–50% of initial rate) and stable pattern of DVocs throughout the remainder of the 2-h test session. Imipramine, but not chlordiazepoxide, displayed antidepressant activity by increasing DVoc rates during this phase of the model. Furthermore, plasma corticosterone levels, a common biomarker of stress and aimed at establishing construct validity, increased over the first 15 min of isolation and decreased thereafter. This pattern is typical of stress-provoking stimuli and reflects a negative feedback process of the glucocorticoid system (Keller-Wood and Dallman, 1984). Collectively, these findings provided preliminary evidence that an anxiety-depression continuum could be modelled in domestic fowl chicks.

The present research sought to extend the predictive and construct validity of the chick anxiety-depression continuum model. To further the predictive validity of the model, separate dose–response studies were performed on chemically diverse and clinically approved anxiolytics (i.e., chlordiazepoxide and clonidine) and antidepressants (i.e., imipramine, maprotiline and fluoxetine). It was predicted that drug probes possessing anxiolytic properties would dose-dependently decrease DVocs in the anxiety phase of the model and that drug probes possessing antidepressant properties would dose-dependently increase DVocs in the depression phase of the model.

In addition to pharmacological manipulations, the paradigm's validity was also evaluated through the assessment of changes in cytokine levels, which is a biological marker of depression. Cytokines are proteins involved in a wide range of immunological, neurophysiological and neuroendocrine functions and are thought to be a major contributing pathological feature underlying the anxiety-depression continuum hypothesis (Castanon, *et al*., 2002; Kim, *et al*., 2007; O'Brien, *et al*., 2004; Schiepers, *et al*., 2005). Indeed, elevated levels of cytokines (e.g., interleukin-1β, -2 and -6 and interferon) have been reported in individuals diagnosed with depression (Kim, *et al*., 2007; Motivala, *et al*., 2005; Pace, *et al*., 2006; Pike and Irwin, 2006; Schiepers, *et al*., 2005). To further the construct validity

of the chick anxiety-depression continuum model, blood plasma was collected following varying lengths of social-separation with interleukin-6 (IL-6) levels quantified via ELISA. It was predicted that increased IL-6 levels would be detected during the depression phase of the model.

Methods

Subjects

Cockerels (*Gallus gallus*; W36; Cal-Maine Foods, Inc., Mendenhall, Mississippi, USA) were received 1-day post hatch and housed in $34 \times 57 \times 40$ cm^3 stainless steel cages with 12–13 chicks per cage. 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.

Drugs

The following drug probes, each with demonstrated anxiolytic and/or antidepressant clinical efficacy, were used in the pharmacological validation experiments: chlordiazepoxide (2.5, 5.0, 10.0 and 15.0 mg/kg), clonidine (0.10, 0.15, 0.20 and 0.25 mg/kg), imipramine (1.0, 3.0, 10.0 and 15.0 mg/kg), maprotiline (2.5, 5.0, 10.0 and 20.0 mg/kg) and fluoxetine (1.0, 5.0, 10.0 and 15.0 mg/kg). The drug doses were selected from pilot studies and previous work showing their ability to alter DVoc rates (Feltenstein and Sufka, 2005; Feltenstein, *et al*., 2004; Sufka, *et al*., 2006; Warnick, *et al*., 2006). Physiological saline (0.9%) or DMSO (for maprotoline and fluoxetine studies) served as the vehicles in the drug probe experiments. For the IL-6 experiment, physiological saline was administered before testing.

Procedure

All experiments were conducted across ages 5 to 7-days post-hatch. The groups in each drug probe experiment formed a 1×5 factorial design (probes tested in isolated chicks) with a hanging control condition (vehicle-treated chicks tested in the presence of two social companions). Sample sizes were $n = 9$–12. Vehicle or a drug was administered i.m. 15 min before testing. The test session involved placing chicks in a Plexiglas test chamber (either in isolation or with two social companions) located in sound attenuating chambers (see Sufka, *et al*., 2006 for complete description) for a 120-min test observation period during which DVocs were collected in 5 min blocks via sound-activated relays. During the test session, animals were monitored for sedative effects that could potentially confound the dependent measures. None of the animals appeared sedated across any of the drug probes at the doses selected. Animals were returned to their home cage following the test session and killed upon completion of the experiment.

The groups in the IL-6 study consisted of a no-test control (No Test), a group of chicks tested with two social companions for 120 min (Social 120), and four groups of chicks tested in isolation at 15, 30, 60 or 120 min (Isolated-15, -30, -60 or -120). Samples sizes were $n = 12$. As before, saline was administered i.m. 15 min before testing and DVocs were recorded in 5-min time blocks. At the appropriate time point, half of the animals (i.e., $n = 6$) from each group were rapidly decapitated and blood was collected in EDTA tubes (BD Vacutainer K3 EDTA) and placed in ice. Following the completion of the day's test session, blood was centrifuged for 15 min at 3000 rpm and plasma was removed. Plasma was stored at −80 °C until analysis. Plasma IL-6 concentrations were determined by an ELISA kit (IL-6 Mouse EIA; ALPCO Diagnostics, Salem, New Hampshire, USA). The assay was conducted according to the manufacturer's instructions. The intra-assay coefficient was 6.05%.

These procedures were approved by the University of Mississippi Institutional Animal Care and Use Committee (Protocol #06-013) and conducted under the ethical guidelines of the American Psychological Association.

Statistical analyses

To compare drug effects across the two phases of the anxiety-depression continuum, it was necessary to convert the DVoc data to a rate per minute function. Thus, the anxiety phase was calculated as total DVocs during the first 5 min time block/5 and the depression phase was calculated as the total DVocs during the 30–120 min time block/90. For analyses, DVoc rates for the anxiety and depression phases of the model were conducted using a one-way ANOVA followed by Fisher's LSD post-hoc tests. Plasma IL-6 concentration data were analysed using one-way ANOVA followed by Fisher's LSD and independent and paired *t*-tests.

Results

Chlordiazepoxide probe

Vehicle-treated chicks tested in the presence of two social companions displayed relatively few DVocs across the 2-h test session, whereas vehicle-treated isolated chicks displayed very large numbers of DVocs that decreased over the first 30 min of the session to about 50% of the initial rate and remained relatively stable thereafter. Chlordiazepoxide decreased DVoc rates in both the anxiety and depression phases of the model (see Figure 1a,b). Consistent with these descriptions, a one-way ANOVA of the isolated groups during the anxiety phase of the model (0–5 min) showed a significant main effect for dose [$F(4, 56) = 15.173$, $P < 0.0001$]. Post-hoc analyses reported that the 10.0 and 15.0 mg/kg doses of chlordiazepoxide produced a significant attenuation (i.e., anxiolytic effect) of

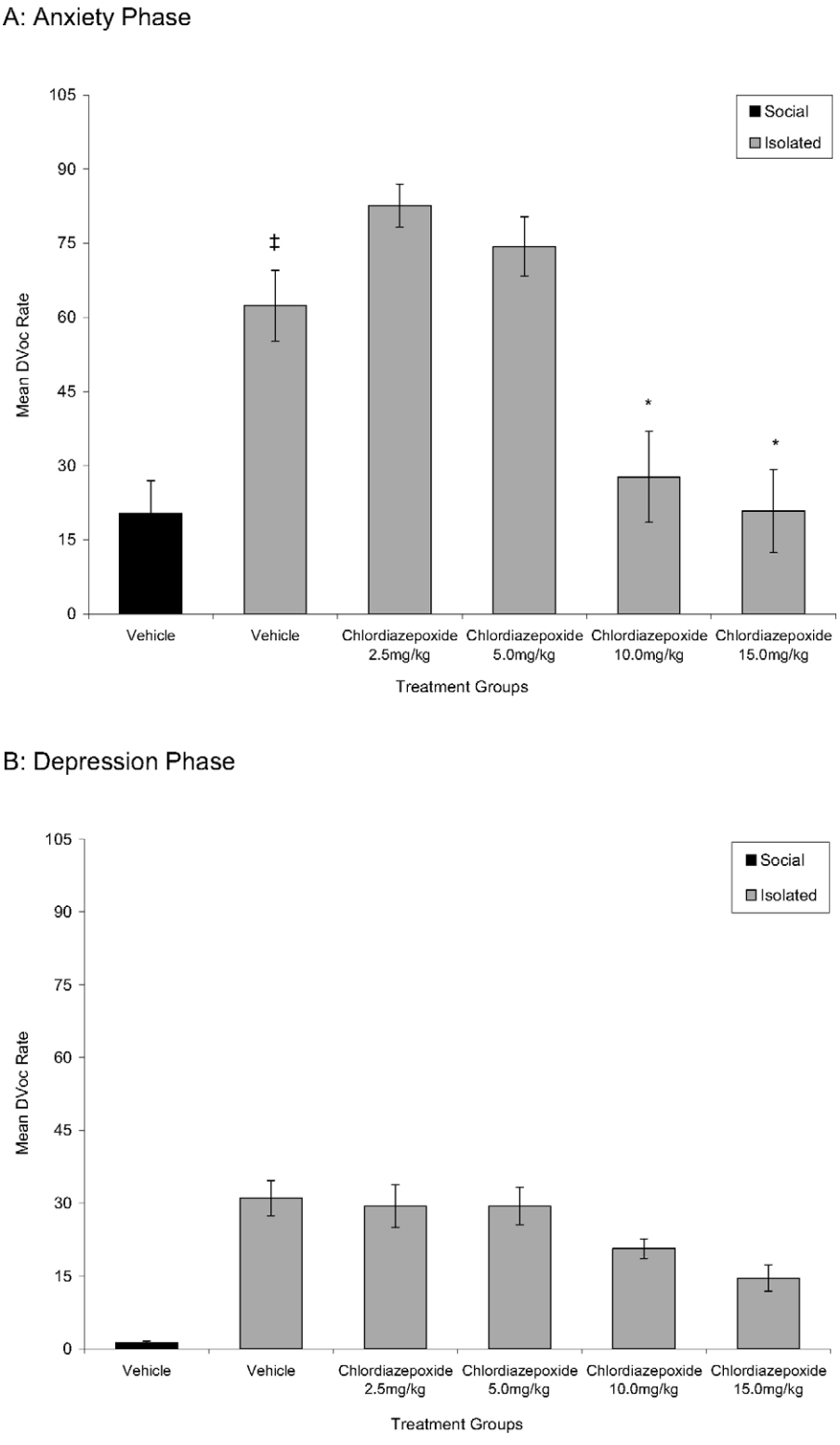

Figure 1 The effects of chlordiazepoxide on DVoc rates during the anxiety phase (minutes 0–5, panel A) and the depression phase (minutes 30–120, panel B) of the test session. Values represent mean ± SEM. ‡indicates significant increase in DVoc rate compared with the vehicle/social condition. *indicates a significant attenuation of DVoc rate compared with the vehicle/isolated condition. All $P^s < 0.05$.

DVoc rates compared with the vehicle-isolated group ($P^s < 0.005$). A one-way ANOVA of the isolated groups during the depression phase of the model (30–120 min) showed a significant main effect for dose [$F(4, 56) = 4.341$, $P < 0.005$]. Post-hoc analyses reported that the 10.0 and 15.0 mg/kg doses of chlordiazepoxide produced a significant attenuation of DVoc rates compared with the vehicle-isolated group ($P^s < 0.025$).

Clonidine probe

As before, vehicle-treated chicks tested in the presence of two social companions displayed relatively few DVocs across the 2-h test session, whereas vehicle-treated isolated chicks displayed very large numbers of DVocs that decreased over the first 30 min of the session to about 50% of the initial rate and remained relatively stable thereafter. Clonidine decreased DVoc rates in only the anxiety phase of the model (see Figure 2a,b). Consistent with these descriptions, a one-way ANOVA of the isolated groups during the anxiety phase of the model (0–5 min) showed a significant main effect for dose [$F(4, 56) = 26.030$, $P < 0.0001$]. Post-hoc analyses reported that all four doses of clonidine produced a significant attenuation (i.e., anxiolytic effect) of DVoc rates compared with the vehicle-isolated group ($P^s < 0.0001$). A one-way ANOVA of the isolated groups during the depression phase of the model (30–120 min) failed to show a significant main effect for dose. No further analyses were conducted on these data.

Imipramine probe

The pattern of DVocs in vehicle-treated social and isolated chicks is consistent with the previous two experiments. Imipramine decreased DVoc rates in the anxiety phase and increased DVoc rates in the depression phase of the model (see Figure 3a, b). Consistent with these observations, a one-way ANOVA of the isolated groups during the anxiety phase of the model (0–5 min) showed a significant main effect for dose [$F(4, 52) = 3.456$, $P < 0.025$]. Post-hoc analyses showed that the 10.0 and 15.0 mg/kg doses of imipramine produced a significant attenuation (i.e., anxiolytic effect) of DVoc rates compared with the vehicle-isolated group ($P^s < 0.05$). A one-way ANOVA of the isolated groups during the depression phase of the model (30–120 min) showed a significant main effect for dose [$F(4, 52) = 5.044$, $P < 0.005$]. Post-hoc analyses reported that the 1.0, 3.0 and 15.0 mg/kg doses of imipramine significantly increased (i.e., antidepressant effect) DVoc rates compared with the vehicle-isolated group ($P^s < 0.005$). The increase in DVoc rates at the 10.0 mg/kg dose was marginally significant ($P = 0.058$).

Maprotiline probe

The pattern of DVocs in vehicle-treated social and isolated chicks is consistent with the previous three experiments. Maprotiline decreased DVoc rates in the anxiety phase and increased DVoc rates in the depression phase of the model (see Figure 4a,b). Consistent with these observations, a one-way ANOVA of the isolated groups during the anxiety phase of the model (0–5 min) showed a significant main effect for dose [$F(4, 59) = 5.384$, $P < 0.0001$]. Post-hoc analyses reported that the 20.0 mg/kg dose of maprotiline produced a significant attenuation (i.e., anxiolytic effect) of DVoc rates compared with the vehicle-isolated group ($P^s < 0.005$). A one-way ANOVA of the isolated groups during the depression phase of the model (30–120 min) showed a significant main effect for dose [$F(4, 59) = 4.872$, $P < 0.005$]. Post-hoc analyses reported that the 2.5, 5.0 and 10.0 mg/kg doses of maprotiline significantly increased (i.e., antidepressant effect) DVoc rates compared with the vehicle-isolated group ($P^s < 0.01$).

Fluoxetine probe

The pattern of DVocs in vehicle-treated social and isolated chicks is consistent with the previous four experiments. Fluoxetine did not affect DVoc rates in the anxiety phase of the model but did increase DVoc rates in the depression phase of the model (see Figure 5a,b). Consistent with these observations, a one-way ANOVA of the isolated groups during the anxiety phase of the model (0–5 min) failed to show a significant main effect for dose. No further analyses were conducted on this data set. A one-way ANOVA of the isolated groups during the depression phase of the model (30–120 min) showed a significant main effect for dose [$F(4, 54) = 2.797$, $P < 0.05$]. Post-hoc analyses reported that the 1.0, 10.0 and 20.0 mg/kg doses of fluoxetine significantly increased (i.e., antidepressant effect) DVoc rates compared with the vehicle-isolated group ($P^s < 0.05$).

Interleukin-6

The pattern of chick DVocs in the anxiety-depression continuum model from the IL-6 study are summarized in Figure 6a. Consistent with the findings from the previous five experiments, chicks tested in the presence of two social companions (open squares) displayed relatively few DVocs across the 2-h test session, whereas isolated chicks (closed symbols) displayed very large numbers of DVocs that decreased over the first 30 min of the session to about 50% of the initial rate and remained relatively stable thereafter. Because of the incomplete factorial design of the study, the two 120-min test groups (social versus isolated) were selected for initial analyses. A two-way ANOVA showed significant main effect for Test Condition [$F(1, 10) = 30.63$, $P < 0.0001$] and a significant main effect for Time [$F(23, 230) = 5.99$, $P < 0.0001$]. The Test Condition × Time interaction term was not significant. A one-way repeated measures ANOVA conducted on DVocs in the Isolated-120 group showed a significant main effect for Time [$F(23, 115) = 4.95$, $P < 0.0001$]. Paired samples t-tests showed significantly lower DVocs for every time block (10–120 min) compared with the first 5-min time block (all $P^s < 0.05$). All other isolated conditions displayed a similar pattern with the

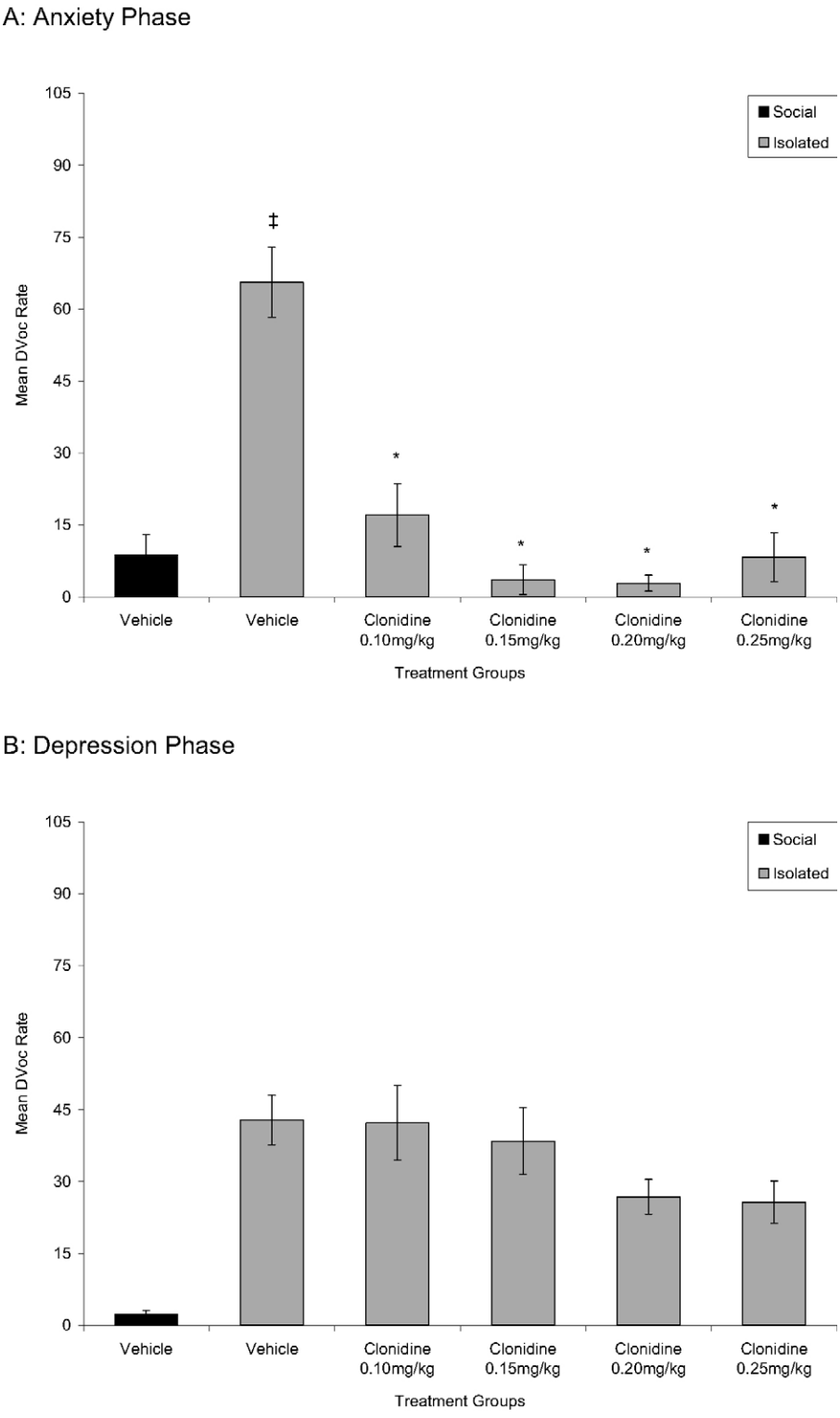

Figure 2 The effects of clonidine on DVoc rates during the anxiety phase (minutes 0–5, panel A) and the depression phase (minutes 30–120, panel B) of the test session. Values represent mean ± SEM. ‡indicates significant increase in DVoc rate compared with the vehicle/social condition. *indicates a significant attenuation of DVocs compared with the vehicle/isolated condition. All $P^s < 0.05$.

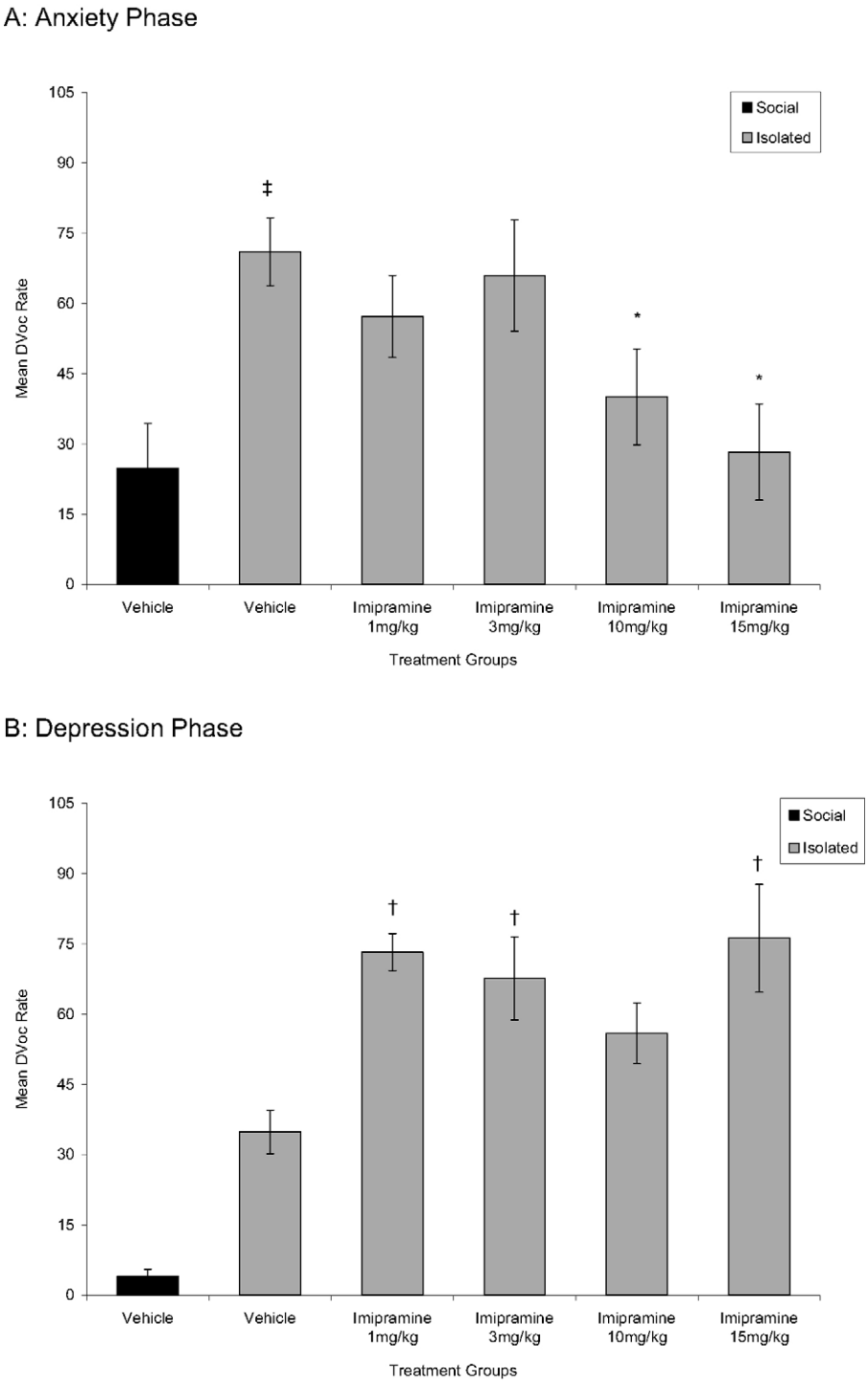

Figure 3 The effects of imipramine on DVoc rates during the anxiety phase (minutes 0–5, panel A) and the depression phase (minutes 30–120, panel B) of the test session. Values represent mean ± SEM. ‡indicates significant increase in DVoc rate compared with the vehicle/social condition. *indicates a significant attenuation of DVocs compared with the vehicle/isolated condition. †indicates a significant increase of DVocs compared with the vehicle/isolated condition. All $P^s < 0.05$.

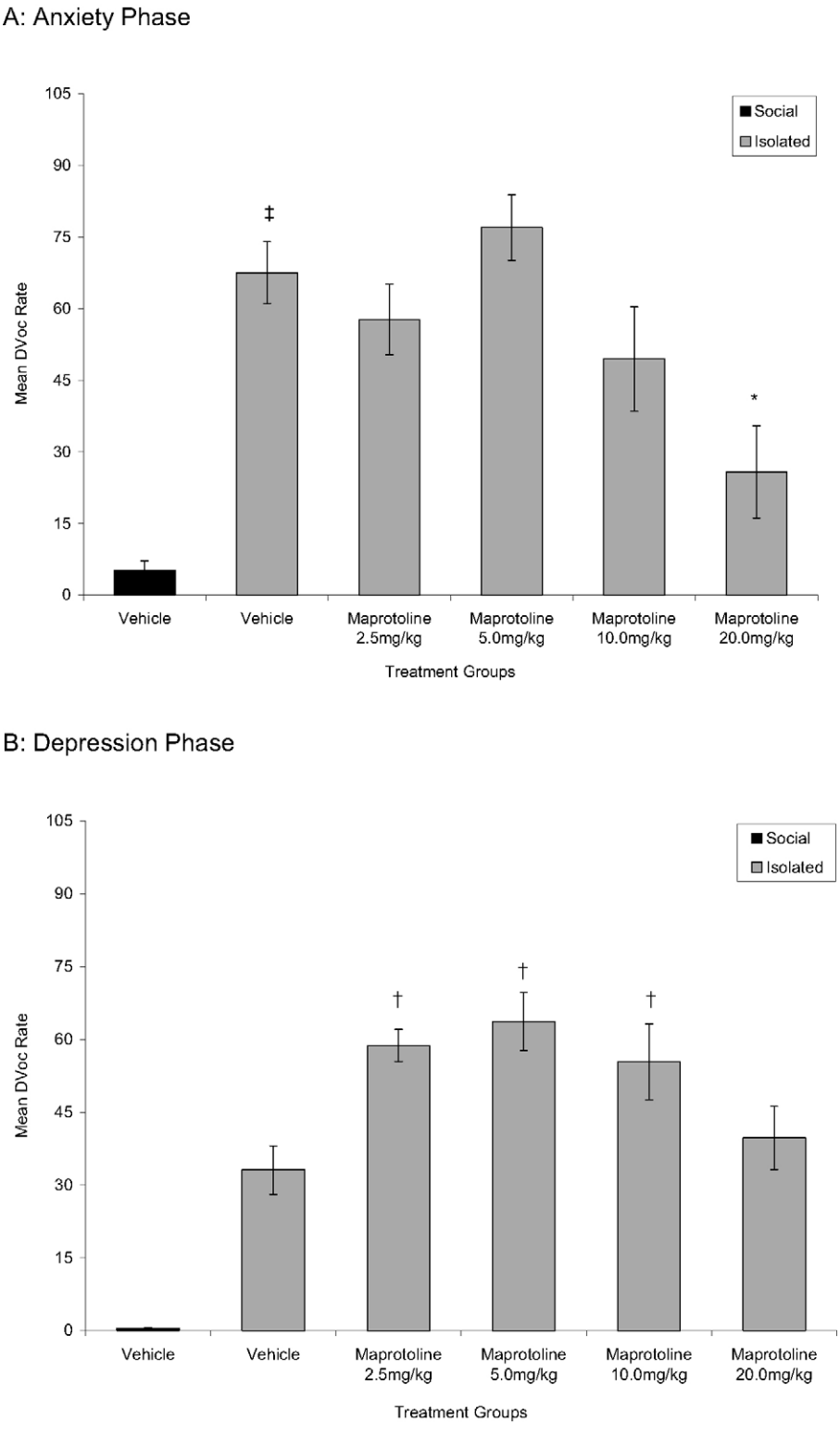

Figure 4 The effects of maprotiline on DVoc rates during the anxiety phase (minutes 0–5, panel A) and the depression phase (minutes 30–120, panel B) of the test session. Values represent mean ± SEM. ‡indicates significant increase in DVoc rate compared with the vehicle/social condition. *indicates a significant attenuation of DVocs compared with the vehicle/isolated condition. †indicates a significant increase of DVocs compared with the vehicle/isolated condition. All $P^s < 0.05$.

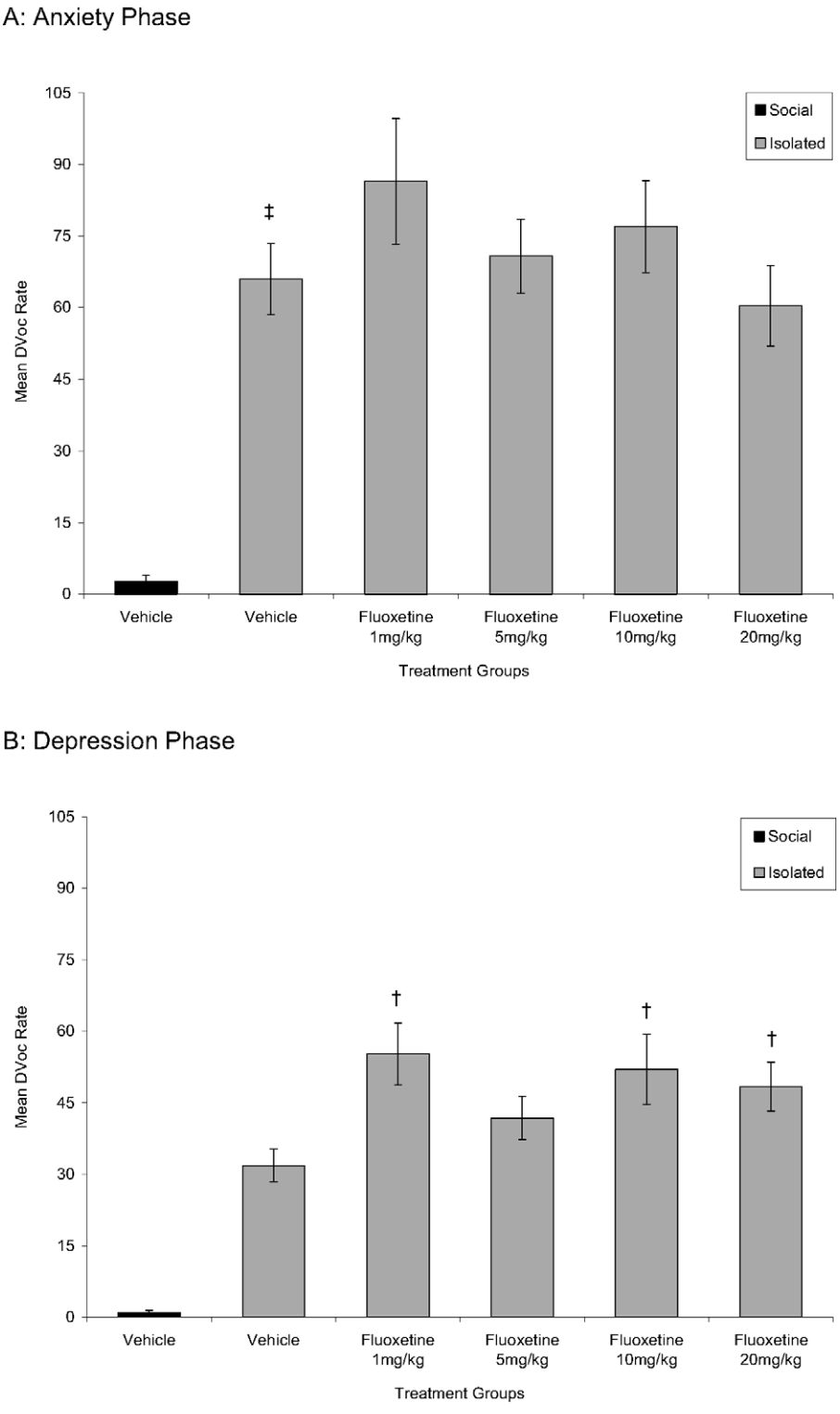

Figure 5 The effects of fluoxetine on DVoc rates during the anxiety phase (minutes 0–5, panel A) and the depression phase (minutes 30–120, panel B) of the test session. Values represent mean ± SEM. ‡indicates significant increase in DVoc rate compared with the vehicle/social condition. †indicates a significant increase of DVocs compared with the vehicle/isolated condition. All $P^s < 0.05$.

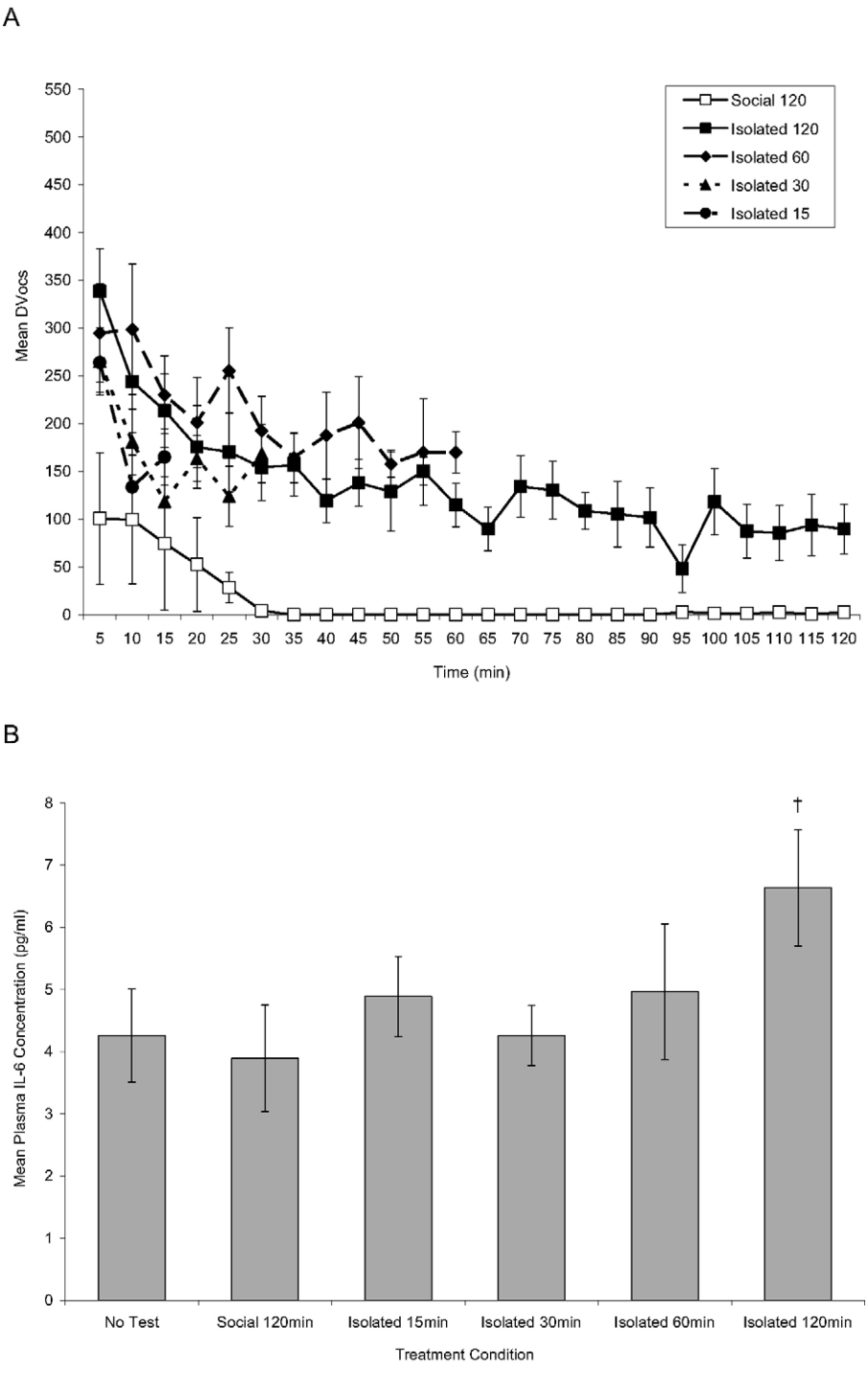

Figure 6 Panel A: The effects of separation stress on DVocs across the 2-h test session. Values represent mean ± SEM for each 5-min time block. Panel B: Plasma interleukin-6 (pg/mL) concentrations. Values represent mean ± SEM. †indicates significant increase in plasma IL-6 compared with the Social-120 min condition ($P < 0.05$).

DVoc frequency decreasing across the test session (all $P^s < 0.05$).

The effects of varying lengths of social isolation stress on mean plasma IL-6 concentrations are summarized in Figure 6b. Plasma IL-6 levels were comparable between the No-Test and the Social-120 min groups. Plasma IL-6 levels were stable across the majority of the isolation test session until an increase in IL-6 levels was observed in the Isolated-120 group. A one-way ANOVA of these data showed a significant main effect for Test Condition [$F(5, 35) = 1.44$, $P = 0.024$]. Post-hoc analyses found no difference in IL-6 levels between the No-Test and Social-120 groups. Furthermore, the Isolated-120 group displayed a significant elevation in IL-6 levels compared with No-Test and Social-120 groups ($P^s < 0.05$).

Discussion

This research sought to further validate the chick anxiety-depression continuum model as a simulation and as a potential pharmacological screen. The experiments consisted of testing the efficacy of clinically established anxiolytics (i.e., chlordiazepoxide and clonidine) and anxiolytic/antidepressants (i.e., imipramine, maprotiline and fluoxetine) in the model and an additional study that quantified plasma IL-6 concentrations, a biomarker of stress, across the test session. In all experiments, vehicle-treated chicks tested with social companions displayed relatively few vocalizations across the test session. On the contrary, vehicle-treated chicks tested in isolation displayed high-DVoc rates that decreased over the first 20–30 min of the test session to about 50% the initial rate and remained relatively stable thereafter. This pattern of DVoc rate is consistent with our previous findings and highlight the anxiety (0–5 min) and depression (30–120 min) phases of the model (Sufka, *et al*., 2006).

All the drug probes, with the exception of fluoxetine, attenuated DVoc rates during the anxiety phase of the model and this drug effect is indicative of an anxiolytic action (Feltenstein and Sufka, 2005; Feltenstein, *et al*., 2004; Sufka, *et al*., 2006; Warnick, *et al*., 2006). The antidepressant drug probes imipramine, maprotiline and fluoxetine increased DVoc rates during the depression phase of the model and this drug effect is indicative of an antidepressant action (Lehr, 1989; Sufka, *et al*., 2006).

The inability of fluoxetine to modulate the anxiety phase of the model is not surprising. Although fluoxetine is used clinically for the treatment of panic disorder (Gorman, *et al*., 1987; Michelson, *et al*., 1998; Michelson, *et al*., 1999), it has been shown to be ineffective in the treatment of situationally bound panic disorder (Uhlenhuth, *et al*., 2000). Thus, the absence of a fluoxetine effect in the anxiety phase of the model appears to be a true negative rather than a false negative and further defines this phase as modelling situationally bound panic disorder.

In the present study, elevated plasma IL-6 concentration was detected after 120 min of social-separation stress. Clinical studies have shown that major depression is associated with an elevation in a number of cytokines, including plasma IL-6 (Kim, *et al*., 2007; Pike and Irwin, 2006). This increased production of cytokines has been linked to changes in glucocorticoid levels that accompany anxiety states (O'Brien, *et al*., 2004). On the basis of our previous research showing chick corticosteroid levels that are elevated early in the test session (Sufka, *et al*., 2006), the increased IL-6 production at the end of the current study's test session appears to mirror the anxiety-depression continuum. The results of cytokine assays in animal models of depression have been equivocal. Although some studies (Dunn, *et al*., 2005; Mormède, *et al*., 2003) failed to show any change in plasma IL-6, other reports (Zhou, *et al*., 1993) did show increased plasma IL-6 concentrations. The ability to detect increased plasma IL-6 concentrations in the chick model sets it apart from standard depression models and provides additional evidence of the model's construct validity.

Clinical studies report that panic disorder plays a key role in subsequently developing depression (Ball, *et al*., 1994). Furthermore, it is not uncommon for depression and panic disorder to be comorbid in patients (Zajecka and Ross, 1995). In the present study, this chick anxiety-depression continuum simulation detected 1) activity in all probes efficacious for situationally bound panic disorder (i.e., clonidine, chlordiazepoxide, imipramine and maprotiline), 2) activity in all probes efficacious for depression (i.e., imipramine, maprotiline and fluoxetine) and 3) elevated levels of the cytokine IL-6. Such similarities in pharmacological sensitivity and neuropathology suggest similarities in the underlying neurobiology (Willner, 1990a). Taken together, these findings further the predictive, face and construct validity of the chick anxiety-depression continuum as a clinical simulation and putative preclinical screening assay.

Although rodent-based assays are the mainstay of behavioural pharmacology research, the use of less common animal models, such as the domestic fowl chick, could offer unique opportunities for researchers. In fact, this avian model possesses three attributes that strongly argue for its adoption as a supplement to rodent models as an early preclinical dual anxiolytic/antidepressant-screening assay. First, using the criteria outlined byWillner (1990a), this assay possesses high utility for an in-vivo screening assay as it 1) uses a lower purchase cost animal compared with rodents ($0.50 a chick), 2) tests at a young age leading to lower total per diem costs, 3) employs a behavioural index that can be automatically recorded, 4) screens for two drug properties in a single test and 5) uses simple experimental designs and statistical analyses. Second, this chick model appears to address each of the National Institutes of Health's 3R policy to Reduce, Refine and Replace animals in research (Office of Laboratory Animal Welfare, 2002; Russell and Burch, 1959). The model reduces the number of purpose-bred research animals because 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 test session rather than two required of an anxiolytic and antidepressant screen. And the model replaces the standard rodent-based

models of anxiety and depression with a phylogenetically lower and, perhaps, less-sentient species. Third, the domestic fowl could provide a novel avenue in the study of topics relevant to psychopharmacology, such as interspecies behavioural genetics (Kas, *et al*., 2007). This appears especially promising as the chick is a species that has been used in diverse fields of study including, among others, social attachment (Panksepp, *et al*., 1978; Warnick, *et al*., 2005), memory (Barber, *et al*., 1998; Cozzutti and Vallortigara, 2001; Crowe and Hamalainen, 2001; Hale and Crowe, 2001; Johnston and Rose, 2001; Salinska, *et al*., 2001; Toukhsati and Rickard, 2001), vestibular function (Aldrich and Peusner, 2002), reward (Yanagihara, *et al*., 2001), feeding (Ando, *et al*., 2001), pain (Bardo and Hughes, 1978; Gentle and Corr, 1995; Hughes and Sufka, 1991; Ito and Böhm, 1986; Roach and Sufka, 2003) and emotion (Lehr, 1989; Panksepp, *et al*., 1991; Sufka, *et al*., 2006).

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