Antidepressant efficacy screening of novel targets in the chick anxiety-depression model
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The chick anxiety-depression model is a hybrid simulation, which may prove useful as an early preclinical dual pharmacological screen for novel therapeutics. Separate dose–response studies were conducted with seven test compounds that have screened positive for antidepressant effects in rodent depression models and included prasterone (5.0–40.0 mg/kg), memantine (2.5–20.0 mg/kg), ketamine (1.0–10.0 mg/kg), mifepristone (50.0–400.0 mg/kg), DOV216,303 (5.0–20.0 mg/kg), CGP36742 (2.5–15.0 mg/kg), and antalarmin (1.0–30.0 mg/kg). Chicks aged 4–6 days post hatch received test compounds intramuscularly 15 min before social separation, in which distress vocalization rates were recorded. High rates of vocalization in the first phase (0–5 min) of social separation seem to model an anxiety-like state and lower rates of vocalization in the second phase (30–60 min) seem to model a depression-like state. Prasterone, memantine, ketamine, and DOV216,303 attenuated and CGP36742 enhanced the pattern of vocalizations in the first phase. Prasterone, ketamine, mifepristone, DOV216,303, and CGP36742 attenuated and memantine and antalarmin enhanced the pattern of vocalizations in the second phase. This pattern of drug effects parallels what clinical data exist, and highlights two important characteristics of this dual-screening assay. For the compounds tested, this chick model identified phase II and III clinical failures (e.g. memantine and antalarmin) and has the potential to reveal possible contraindications of compounds (i.e. CGP36742) in cases where anxiety symptoms are concomitant with a depressive episode. 


Keywords: antidepressant screen, anxiolytic screen, domestic fowl, hybrid model, separation stress

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Introduction

Numerous animal simulations and drug screening assays (Willner, 1991a) exist for various neuropsychiatric syndromes including, among others, anxiety and depression (Fuchs and Flügge, 2006). Typically, these paradigms were designed to model a predominant feature of a syndrome such as innate or conditioned fear in many anxiety models (Green and Hodges, 1991) and ‘behavioral despair’ or learned helplessness in depression models (Willner, 1991b). Animal models have been beneficial in understanding genetic and environmental contributions to (Henn and Vollmayr, 2005; Pryce et al., 2005) and underlying neural and chemical substrates of (Dunn et al., 2005) clinical syndromes and, in many instances, have revealed novel targets for psychotherapeutics (McArthur and Borsini, 2006).

Two issues have been raised concerning the validity of such animal models. The first centers on the use of single endophenotypes to characterize a given model, where the clinical symptoms are often diverse and complex (Frazier and Morilik, 2003; Matthews et al., 2005; Van der Staay, 2006). In response, researchers have used a combination of behavioral (e.g. grooming), physiological (e.g. thermoregulation), and biological (e.g. corticosterone) measures to better characterize a given model. The second concerns the validity of *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (American Psychiatric Association, 2000) clinical categories upon which animal models are based, noting that many neuropsychiatric disorders fail to form discrete and separable syndromes. This is particularly true for anxiety and depression, where these two disorders share many symptoms (Baldwin et al., 2002; Watson, 2005) and present comorbidity rates ranging from 50 to 90% (Rivas-Vázquez et al., 2004; Kessler et al., 2005). In response, researchers have suggested several approaches to combining or ‘hybridizing’ animal models to emphasize the observed relatedness of certain *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision clinical categories (Kalouet et al., 2007, 2008a,b; Veen et al., 2008).

Recent research has developed a hybrid procedure in which anxiety and depression have been modeled within a single paradigm (Sufka et al., 2006; Warnick et al., 2009). The anxiety-like portion of the model is based on studies by Pankspe and others that show brief social-separation stress produce high rates of distress vocalizations in chicks and that these calls are reliably reduced by...
anxiolytic compounds (Panksepp et al., 1978, 1980; Rossi et al., 1983; Panksepp, 2003; also see Feltenstein and Sufka, 2008). A recent study shows that this portion of the 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 trazadone), suggesting that the simulation, with its symptom onset being rapid, intense and brief with clear etiological origins, most closely resembles situational panic disorder (Warnick et al., 2006).

The depression-like portion of the model is based on studies by Lehr (1989) and others (Panksepp et al., 1991) in which a marked, temporal-dependent reduction in vocalization rates occurs in chicks subjected to a 2-h isolation period. Lehr (1989) argued that the ‘ethodynamic slope’ fits well with early ‘protest’ and later ‘resignation’ phases that fit well with models of depression (Katz, 1981). Further, a wide variety of antidepressant drugs (n = 11) reversed this decline in vocalization rates, by enhancing total distress calls during the second hour of isolation (i.e. resignation phase), whereas compounds lacking antidepressant activity (n = 22) did not (Lehr, 1989) or, in the case of sedative-hypnotic compounds, lowered rates even further. Many of these latter compounds possess anxiolytic action but such effects in the ‘protest’ phase of the model were overlooked.

The hybrid chick anxiety-depression model continuously measures separation-induced vocalizations in 4–6 days old chicks (Sufka et al., 2006). Isolated chicks display high rates of vocalizations during the initial 5 min block (first or anxiety-like phase) that gradually decline to about 50% the initial rate at about 25–30 min into the isolation period (second or depression-like phase) and remain stable for the remainder of the session. The two phases are pharmacologically dissociable in which the anxiolytics chlordiazepoxide and clonidine attenuate the high vocalization rates during the first phase but are without effects thereafter. In contrast, the antidepressants imipramine, maprotiline, and fluoxetine attenuate the decline in vocalization rates during the second phase (Warnick et al., 2009). Interestingly, both imipramine and maprotiline, which have anti-panic effects, are also effective in attenuating vocalizations in the first phase of the model. Furthermore, plasma levels of corticosterone (Sufka et al., 2006) and interleukin-6 (Warnick et al., 2009), both of which are biomarkers of stress, reveal patterns that parallel those found in human anxiety and depression syndromes.

The chick anxiety-depression model has been shown to possess varying degrees of face, predictive and construct validity as a simulation (for review, see Warnick and Sufka, 2008). However, for the model to be useful as an early preclinical screening assay, it will be necessary to determine its ability to identify efficacious compounds acting at novel targets, particularly for depression as this area represents a major focus of current drug discovery efforts (Bosker et al., 2004; Norman and Burrows, 2007). Such therapeutic targets have expanded well beyond monoamine manipulations, to include androgen precursors, N-methyl-D-aspartic acid (NMDA) antagonists, glucocorticoid antagonists, γ-amino butyric acid-B (GABA_B) antagonists, and corticotropin-releasing factor-1 (CRF_1) antagonists, among others. Even within the classic pharmacological targets, many compounds being developed have mechanisms of action that are more sophisticated than earlier drug class prototypes such as monoamine triple reuptake inhibition. Thus, the main objective of this study was to evaluate the ability of the chick anxiety-depression model to correctly identify the efficacy of a set of potential novel antidepressants that have each passed preclinical screening tests in traditional rodent models of depression and, in some instances, have gone into clinical trials, with varying results.

**Methods**

**Subjects and housing procedures**

Cockerels (Gallus gallus; W36; Cal-Maine Foods Inc., Mendenhall, Mississippi, USA) were received 1-day posthatch and housed in 34 × 57 × 40 cm stainless steel cages with 12–13 chicks per cage. Food (Purina Start and Grow, St. Louis, Missouri, USA) and water were freely available through 1-quart gravity-fed feeders and waterers. Room temperature was maintained at 29 ± 1°C and overhead illumination was maintained on a 12:12 h light–dark cycle. Daily maintenance involved refilling waterers and feeders, and replacement of tray liners.

**Apparatus**

The six-unit testing apparatus consisted of Plexiglas viewing chambers (25 × 25 × 22 cm) located in sound-attenuating enclosures (for details, see Sufka et al., 2006). Miniature video cameras mounted at floor level outside the Plexiglas chambers permitted televised display of the test session. Distress vocalizations were monitored by microphones affixed through the Plexiglas chambers with input signals routed into a custom-designed software program that continuously recorded distress calls throughout the session.

**Test compounds**

This study consisted of seven separate dose–response studies. The compounds tested, all of which have been shown to be efficacious in preclinical tests in depression models, were the androgen precursor prasterone (Wolkowitz et al., 1999), the NMDA antagonist memantine (Kos and Popik, 2005; Zarate et al., 2006a), the NMDA antagonist ketamine (Zarate et al., 2006b), the glucocorticoid antagonist mifepristone (Belenoff et al., 2002), the monoamine triple reuptake inhibitor DORV216,303 (Skolnick et al., 2006), the GABA_B receptor antagonist...
CGP36742 (Nowak et al., 2006), and the CRF₄ receptor antagonist antalarmin (Jurtkiewicz et al., 2005).

Test compounds were dissolved in a 75% dimethyl sulfoxide and 25% physiological (0.9%) saline solution except for antalarmin, which was dissolved in a 10% cremaphor, 5% ethanol, and 85% deionized water solution and CGP36742, which was dissolved in physiological (0.9%) saline. Doses were based on efficacy data in rodent models of depression (prasterone: 5.0, 10.0, 20.0, and 40.0 mg/kg; memantine: 2.5, 5.0, 10.0, and 20.0 mg/kg; ketamine: 1.0, 2.5, 5.0, and 10.0 mg/kg; mifepristone: 50.0, 100.0, 200.0, and 400.0 mg/kg; DOV216,303: 5, 10, 15, and 20 mg/kg; CGP36742: 2.5, 5.0, 10.0, and 15.0 mg/kg; and antalarmin: 1.0, 3.0, 10.0, and 30.0 mg/kg).

Antalarmin and CGP36742 were synthesized on site. The purity (> 99.5%) and composition of both antalarmin and CGP36742 were in complete agreement with earlier literature reports. All other compounds were obtained from Sigma (St. Louis, Missouri, USA) except for DOV216,303, which was generously donated by DOV Pharmaceutical Inc. (Somerset, New Jersey, USA).

Starting materials and reagents for antalarmin and CGP36742 were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, Missouri, USA) and used without further purification. Reactions requiring anhydrous conditions were carried out under an argon atmosphere using conventional flame-dried glassware protocols. ¹H and ¹³C nuclear magnetic resonance spectra were recorded on a Bruker DRX500 spectrometer (Bruker Optics, Billerica, Massachusetts, USA) and referenced to residual proton or carbon signal of solvent. Mass spectral data were determined by electrospray ionization (ESI)+ or ESI− analysis using a Waters ZQ Mass Spectrometer (Waters Corp., Milford, Massachusetts, USA). Combustion analysis was performed using a PerkinElmer 2400 Series II CHN Analyzer (PerkinElmer, Waltham Massachusetts, USA).

Antalarmin (N-butyl-N-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrrolo,3-d]pyrimidin-4-yl)amine) was synthesized in a seven-step sequence from readily available precursors (Greiner et al., 2002). The product was crystallized from acetonitrile–water to afford translucent crystals; melting point = 85.9–86.1°C; high-resolution mass spectrometry m/z expected: 379.2862; found: 379.2878. Analysis: calculated for C₂₂H₃₃N₄: 54.74% C, 7.74% H, 15.42% N; found: 54.60% C, 7.84% H, 15.34% N.

The GABA₅ antagonist CGP36742 was synthesized in a modified three-step sequence from commercially available triethylphosphonate (Froestl et al., 1995; Petnehazy et al., 2003) and characterized as the hydrochloride salt, white powder, melting point = 197–198°C; mass spectrometry (ESI+) m/z 180 [molecular ion (M)+1]. Analysis: calculated for C₇H₁₉ClN₂O₂P: expected: C 38.99, H 8.88, N 6.49; found: C 38.90, H 8.79, N 6.44.

**Procedure**

All dose–response experiments were conducted at ages 4–6 days posthatch. Sample sizes were n = 9–12. The first three experiments included a vehicle-social control group to illustrate the robust isolation stress effect but this discontinued thereafter, as determination of significant dose effects is performed against the vehicle-isolated group. Compounds were administered in a volume of 1 ml/kg intramuscularly 15 min before testing. The session involved placing chicks into observation chambers during which distress vocalizations were recorded. Animals were returned to their home cages after the test session and euthanized upon completion of the experiment. These procedures have been approved by the University of Mississippi Institutional Animal Care and Use Committee (protocol #06–013).

**Data analyses**

To highlight the change in distress vocalizations across the two phases (and lengths) of the anxiety-depression model, data were converted into a rate/min function. The first phase was calculated as total distress vocalizations during the first 5 min isolation period divided by 5 and the second phase was calculated as the total distress vocalizations during the 30–60 min isolation period divided by 30. Distress vocalization rates were analyzed using two-way and one-way analyses of variance (ANOVA), where appropriate, independent t-tests and Fisher’s least significant difference post-hoc tests.

**Results**

A representative dataset from the prasterone study, highlighting the pattern of vocalizations in vehicle-isolated and vehicle-social (i.e. chicks tested with two social companions) groups is summarized in Fig. 1. Vehicle-social treated chicks displayed few vocalizations across the test session. In contrast, vehicle-isolated chicks displayed high vocalization rates during the initial 5 min block that gradually declined over the next 15–20 min isolation period and then stabilized at about 40% the initial rate throughout the remainder of the test period. A two-way repeated-measures ANOVA on these data revealed significant main effects for treatment [F(1,22) = 104.30, P < 0.001], time [F(11,242) = 16.24, P < 0.001], and a significant treatment × time interaction [F(11,242) = 10.20, P < 0.001]. A comparison of vocalization rates in isolated chicks across the two phases of the separation-stress response yielded mean vocalization rates of 71.5/min (SD = 17.6) during the first phase and 26.6/min (SD = 9.7) during the second phase. A paired t-test for these data revealed a significant phase difference in vocalization rates [t(11) = 10.93, P < 0.001].
The dose–response effects of prasterone on vocalization rates across the two phases of the separation-stress response are summarized in Fig. 2. The two lowest doses of prasterone attenuated the high vocalization rates in the first phase, whereas the highest dose of prasterone attenuated the decline in vocalization rates in the second phase. Consistent with these observations, a one-way ANOVA of vocalization rates revealed a significant dose effect in the first phase of the model \(F(4,59) = 4.35, \ P < 0.005\). Post-hoc analyses showed that mean vocalization rates were significantly lower in the 5 and 10 mg/kg dose groups compared with the vehicle group \((P < 0.05)\). A one-way ANOVA on distress vocalization rates for the second phase of the model also revealed an almost significant dose effect \(F(4,59) = 2.46, \ P = 0.056\). Post-hoc analyses for this phase showed that distress vocalizations in the 40 mg/kg group were significantly higher than the vehicle group \((P < 0.05)\).

The dose–response effects of memantine on vocalization rates across the two phases of the separation-stress response are summarized in Fig. 3. The three highest doses of memantine attenuated the high vocalization rates during the first phase. In the second phase, memantine seemed to enhance the decline in vocalization rates. Consistent with these observations, a one-way ANOVA of these data revealed a significant dose effect in the first phase of the model \(F(4,59) = 2.92, \ P < 0.05\). Post-hoc analyses showed that mean vocalization rates were significantly lower in the 5, 10, and 20 mg/kg dose groups compared with the vehicle group \((P < 0.05)\). A one-way ANOVA on vocalization rates during the second phase also revealed a significant dose effect \(F(4,59) = 3.52, \ P < 0.025\). Post-hoc analyses showed that mean vocalization rates in the 5 and 20 mg/kg groups were significantly lower than the vehicle group \((P < 0.05)\).

The dose–response effects of ketamine on vocalization rates across the two phases of the separation-stress response...
response are summarized in Fig. 4. The highest dose of ketamine attenuated the high vocalization rates in the first phase and attenuated the decline of vocalization rates in the second phase of the model. Consistent with these observations, a one-way ANOVA of these data revealed a significant dose effect in the first phase \[ F(4,55) = 11.31, \ p < 0.001 \]. Post-hoc analyses showed that mean vocalization rates were significantly lower in the 10 mg/kg dose group compared with the vehicle group \( P < 0.001 \). A one-way ANOVA on vocalization rates also revealed a significant dose effect during the second phase of the model \[ F(4,55) = 2.57, \ p < 0.05 \]. Post-hoc analyses showed that mean vocalization rates in the 10 mg/kg group was significantly higher than the vehicle group \( P < 0.05 \).

The dose–response effects of mifepristone on vocalization rates across the two phases of the separation-stress response are summarized in Fig. 5. Mifepristone did not affect the high rates of vocalizations in the first phase of the model. In the second phase of the model, however, mifepristone produced an inverted U-shaped dose–response function with the intermediate dose attenuating the decline in vocalization rates. Consistent with these observations, a one-way ANOVA of these data failed to reveal a significant dose effect during the first phase and revealed a significant dose effect in the second phase of the model \[ F(4,53) = 3.15, \ p < 0.025 \]. Post-hoc analyses in this latter phase showed that mean vocalization rate in the 50 mg/kg group was significantly higher than the vehicle group \( P < 0.05 \).

The dose–response effects of DOV216,303 on vocalization rates across the two phases of the separation-stress response are summarized in Fig. 6. The two highest doses of DOV216,303 attenuated the high vocalization rates during the first phase, whereas the intermediate dose attenuated the decline in vocalization rates in the second phase of the model. Consistent with these observations, a one-way ANOVA of these data revealed a significant dose effect during the first phase of the model \[ F(4,55) = 5.09, \ p < 0.005 \]. Post-hoc analyses showed that mean vocalization rates were significantly lower in the 15 and 20 mg/kg dose groups compared with the vehicle group \( P < 0.05 \). Interestingly, a one-way ANOVA on vocalization rates during the second phase of the model failed to reveal a significant dose effect \[ F(4,55) = 1.16, \ p = \text{NS} \], despite the post-hoc findings showing that the 10 mg/kg dose significantly attenuated the decline in vocalization rates during the second phase of the model \( P < 0.05 \).

The dose–response effects of CGP36742 on vocalization rates across the two phases of the separation-stress response are summarized in Fig. 7. CGP36742 tended to increase vocalization rates in both phases of the model. A one-way ANOVA of these data during the first phase revealed a significant dose effect \[ F(4,50) = 3.767, \ p < 0.01 \]. Post-hoc analyses showed that vocalization rates were significantly higher in the 5 and 15 mg/kg dose groups compared with the vehicle group \( P < 0.05 \). A one-way ANOVA on vocalization rates during the second phase also revealed a significant dose effect \[ F(4,50) = 3.343, \ p < 0.025 \]. Post-hoc analyses showed
The effects of DOV216,303 on vocalization rates for the first and second phases in the chick anxiety-depression model. Values represent mean ± SEM. *Significant decrease in vocalization rates compared with the vehicle group. **Significant increase in vocalization rates compared with the vehicle group. All P<0.05.

The effects of CGP36742 on vocalization rates for the first and second phases in the chick anxiety-depression model. Values represent mean ± SEM. **Significant increase in vocalization rates compared with the vehicle group. All P<0.05.

The effects of antalarmin on vocalization rates for the first and second phases in the chick anxiety-depression model. Values represent mean ± SEM.

Response are summarized in Fig. 8. Antalarmin did not affect vocalization rates in either phase of the model. Consistent with these observations, one-way ANOVAs failed to reveal a significant dose effect in either phase.

Discussion
The primary objective of this research was to assess further the predictive validity of the chick anxiety-depression model as a pharmacological screening assay. Earlier studies have shown that this dual-screen is sensitive to a wide range of currently approved anxiolytic and antidepressant compounds (Lehn, 1989; Panksepp et al., 1991; Wamick et al., 2006, 2009). The ability to correctly identify the success or failure of a compound’s outcome in phase II and III clinical trials will not only add to the predictive validity of the model as a pharmacological screen, but it will also strengthen the construct validity of the model as a hybrid simulation of anxiety-depressive disorders. The seven test compounds selected for screening in this study represent a diverse set of drugs acting at novel targets, each of which have shown antidepressant activity in rodent models of depression and, in some cases, have entered clinical trials with varying degrees of success.

Vehicle-social chicks displayed relatively few, if any, vocalizations across the test session. This is not surprising as distress vocalizations are a species-specific response to reestablish social contact with conspecifics. In contrast, vehicle-isolated chicks displayed high rates of distress vocalizations in the first phase of the model that declined approximately 60% as chicks entered into the second phase of the model. Four of the test compounds possessed

that the 5 and 15 mg/kg groups significantly attenuated the decline in vocalization rates compared with the vehicle group (P < 0.025).

The dose–response effects of antalarmin on vocalization rates across the two phases of the separation-stress
anxiolytic-like activity as evidenced by attenuation of vocalizations in the first phase (i.e. anxiety-like) of the model and included prasterone, memantine, ketamine, and DOV216,303. CGP36742 possessed anxiogenic-like activity as illustrated by increasing vocalization rates in the anxiety phase of the model. Mifepristone and antalarmin were inactive in the anxiety phase of the model. Five of the test compounds possessed antidepressant-like activity as evidenced by the attenuation of the decline in vocalization rates in the second phase (i.e. depression-like) and included prasterone, ketamine, mifepristone, CGP36742, and DOV216,303. Memantine and antalarmin did not affect vocalization rates in a manner indicative of possessing an antidepressant-like effect. The effects of each of these compounds in the chick anxiety-depression model are discussed in the context of their known properties in rodent models and, in some instances, human clinical trials.

Prasterone is a testosterone precursor, which is available as an over-the-counter hormone replacement therapeutic. Prasterone has shown anxiolytic-like (Melchior and Ritzmann, 1994) and antidepressant-like (Garcia et al., 2008) activities in rodent models. More importantly, prasterone has shown success in clinical trials for the treatment of major depression (Wolkowitz et al., 1999). We were unable to find any reports of putative anxiolytic effects of prasterone in clinical studies. Based on these published findings, the antidepressant-like effects observed in the chick anxiety-depression model, like the effect seen in rodent models, represents a correct detection of prasterone’s clinical properties. The anxiolytic-like findings in the chick model, along with existing rodent data, suggest that prasterone may also possess anxiolytic properties in clinical populations.

Many NMDA antagonists possess side effects that limit their therapeutic potential. Nevertheless, both memantine and ketamine have shown antidepressant-like activity in rodent models (Kos and Popik, 2005; Garcia et al., 2008) and have been examined for efficacy in clinical trials. The outcome of these trials has been interesting, particularly in the light of the current findings in the chick model: ketamine shows evidence of antidepressant activity in clinical populations (Zarate et al., 2006a), whereas memantine does not (Zarate et al., 2006b). These clinical findings match the screening results for ketamine and memantine in the depression-like phase of the chick model. Given that rodent depression models produced a false positive for memantine and the chick anxiety-depression model did not, one could argue this chick paradigm is a better predictor of phase II and III clinical failures. It is unclear how to interpret the anxiolytic-like effects of ketamine and memantine in the chick model, as NMDA receptor antagonists are not generally considered to be a target of interest in anxiolytic drug development strategies, and we have been unable to locate any clinical trials of these compounds for such conditions.

Mifepristone has been primarily used as an emergency contraceptive in humans. Glucocorticoid antagonists have been shown to possess antidepressant-like activity in a rodent depression model (Bachmann et al., 2005), and mifepristone has shown success in clinical trials for the treatment of major depression (Belanoff et al., 2002). Based on these published findings, the antidepressant-like effects observed in the chick anxiety-depression model, like the effect seen in the rodent model, represent a correct detection of the clinical properties of mifepristone. Glucocorticoid antagonists have shown anxiolytic-like properties in the elevated plus maze (Korte et al., 1995). In this study, mifepristone failed to show such effects in the anxiety-like phase of the chick model. Given that we are unable to find any reports of putative anxiolytic effects of mifepristone in clinical studies, it remains to be determined whether this specific finding represents a false negative in the chick model or a correct response.

Currently, first-line pharmacological treatments for depression act to inhibit norepinephrine and/or serotonin transporters. Recently, triple reuptake inhibitors have been developed and these show antidepressant-like activity in rodent models of depression (Skolnick et al., 2003). Moreover, compounds such as DOV216,303 show antidepressant activity comparable with citalopram in phase II clinical trials (Skolnick et al., 2006). Based on these published findings, the antidepressant-like effects observed in the chick anxiety-depression model, like those seen in rodent models, represent a correct detection of the clinical properties of DOV216,303.

CGP36742 is one of several GABA	extsubscript{A} receptors that show antidepressant-like activity in rodent models of depression (Nowak et al., 2006). CGP36742 is well-tolerated and currently used in clinical trials for the treatment of cognitive impairment (Froestl et al., 2004). To our knowledge, there are no clinical data on the antidepressant activity of CGP36742 or other GABA	extsubscript{A} receptor antagonists. Thus, it is unknown whether the antidepressant-like activity of CGP36742 in the chick anxiety-depression model is a correct or incorrect response. However, it should be noted that CGP36742 did show an anxiogenic-like effect in the chick assay. This is not surprising as many anxiolytics either directly or indirectly act to elevate GABA neurotransmission. Collectively, these data suggest that CGP36742 and other GABA	extsubscript{A} antagonists would likely fail clinical trials in populations that copresent significant anxiety-like symptoms, and highlight a significant feature of this chick dual-screening assay by showing that the model may detect a drug contraindication.

CRF	extsubscript{1} receptor antagonists have shown equivocal results in various rodent models of depression (Nielsen et al., 2004; Jutkiewicz et al., 2005). Despite these mixed findings, CRF	extsubscript{1} receptor antagonists were moved into early clinical
trials for depression (Zobel et al., 2000) but have been discontinued after showing signs of toxicity (Schechter et al., 2005). Despite this setback, the search for nontoxic CRF1 antagonists continues. New clinical trials suggest that the nonpeptide CRF1 antagonist NBI-30775/R121919 shows antidepressant effects equivalent to paroxetine (Holsboer and Ising, 2008). Further, a new clinical trial of GSK561679 is currently under way for treating social anxiety. However, we could not locate any current human trials of antalarmin for either anxiety or depression. In the chick model, antalarmin failed to show either anxiolytic-like or antidepressant-like activity. Whether the clinical trials of antalarmin were abandoned because, exclusively, of toxicity issues or also lack of efficacy, the chick model nonetheless correctly killed a pharmaceutical lead. Like the differences in memantine and ketamine outcomes in the chick model, it would be interesting to see how NBI-30775/R121919 performs in the model. Finally, we would predict that GSK561679 would have little effect in modulating the anxiety-like phase of the model as the chick social-separation stress model purports to simulate panic disorder, not social anxiety.

Conclusion

Rodent-based assays continue to be the mainstay of behavioral pharmacology research. However, the chick anxiety-depression model possesses a number of attributes that warrant consideration as an early preclinical dual-screening assay. First, from this set of experiments, the model was able to identify (i) novel antidepressant compounds that demonstrate efficacy in early clinical trials (i.e. prasterone, ketamine, mifepristone, and DOV216,303), (ii) purported antidepressant compounds that have failed in early clinical trials (i.e. memantine and antalarmin), and (iii) an antidepressant compound that exacerbates anxiety-like behavior (CGP36742), and thus may be contraindicated where depression and anxiety symptoms are comorbid. Where this chick model differs from the rodent depression model, then, is in avoiding two false positives as an antidepressant screening and offering additional efficacy data for compounds with potential anxiolytic-like effects.

Second, using the criteria outlined by Willner (1991a), this assay possesses high utility for an in-vivo screening assay as it (i) uses a lower purchase cost animal compared with rodents ($0.50 a chick), (ii) tests at a young age leading to lower total per diem costs, (iii) uses a behavioral index that can be automatically recorded, (iv) screens for two drug properties in a single test, and (v) uses simple experimental designs and statistical analyses.

Third, this chick model seems to address each of the National Institutes of Health’s 3R policy to ‘reduce’, ‘refine’, and ‘replace’ animals in research as detailed by Russell and Burch (1959). The model reduces the number of purpose-bred research animals as male chicks are a byproduct 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. Finally, the model replaces the standard rodent-based models of anxiety and depression with a phylogenetically lower and, perhaps, less sentient species (Warnick and Sufka, 2008).

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