Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 77 (2004) 221–226
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The chick separation stress paradigm: a validation study

Matt W. Feltenstein[a], Jason E. Warnick[a], Amanda N. Guth[a], Kenneth J. Sufka[a,b,c,*]

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

Received 30 May 2003; received in revised form 20 August 2003; accepted 17 October 2003

Abstract

To expand the generalizability of the chick separation stress paradigm as a high-throughput anxiolytic screen, six positive drug probes (doses in mg/kg: meprobamate 15–120, pentobarbital 2.5–20.0, chlordiazepoxide 2.5–15.0, buspirone 2.5–10.0, imipramine 1–15, and clonidine 0.10–0.25) and five negative drug probes (amphetamine 0.5–4.0, scopolamine 0.2–1.6, caffeine 5–20, chlorpromazine 1–30, and haloperidol 0.03–1.00) were evaluated in the test. Seven-day-old chicks received intramuscular injections of either vehicle or drug probe 15 min prior to tests in either a mirror (low-stress) or a no-mirror (high-stress) condition for a 3-min observation period. The dependent measures were distress vocalizations to index separation stress and sleep onset latency to index sedation. All positive drug probes attenuated distress vocalizations in a dose-dependent manner, except buspirone. All positive drug probes affected sleep onset latency in a dose-dependent manner, except buspirone and imipramine. In all cases, the anxiolytic-like effect of positive drug probes was greater than its sedative effect. None of the negative drug probes affected either distress vocalizations or sleep onset latency, except for the highest dose of amphetamine, which caused pronounced stereotypy. These findings demonstrate that this anxiolytic screen is sensitive to a wide range of positive pharmacological probes and insensitive to a wide range of negative pharmacological probes.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Anxiety; Domestic fowl; Separation stress; Anxiolytic screen; Validation

1. Introduction

The chick separation stress paradigm has traditionally been used as a biobehavioral assay to study attachment (Panksepp et al., 1980; Sufka et al., 1994). In recent years, considerable research has focused on developing the procedure as an anxiolytic screen. As an anxiolytic screen, the paradigm has construct validity (Sufka and Weed, 1994) as well as predictive validity for various benzodiazepine compounds (Watson and Sufka, 1996; Watson et al., 1999). Furthermore, the paradigm has been used to screen putative anxiolytic activity of extracts and isolated compounds derived from botanical products (Sufka et al., 2001; Smith et al., 2001; Feltenstein et al., 2003a). However, the generalizability of this paradigm has not been extended to traditional nonbenzodiazepine anxiolytic classes, nor is it known if the paradigm is sensitive to false positives.

To further validate the chick separation stress paradigm as a high-throughput anxiolytic screen, this study examined the effects of six known anxiolytics that have shown activity in other animal models (i.e., positive controls; see Millan, 2003, for a review), including meprobamate (Geller and Seifter, 1960; Vogel et al., 1971; Crawley, 1981; Howard et al., 1982; De Vry et al., 1993), pentobarbital (Geller and Seifter, 1960; Vogel et al., 1971; Howard et al., 1982; De Vry et al., 1993), chlordiazepoxide (Crawley, 1981; Howard et al., 1982; Salt and Taberner, 1984; Pellow et al., 1985; Lecci et al., 1990; De Vry et al., 1993; King et al., 2002), buspirone (McCloskey et al., 1987; Lecci et al., 1990; Rowan et al., 1990; De Vry et al., 1993; Molewijk et al., 1995; Shimada et al., 1995; Graeff et al., 1998; King et al., 2002), imipramine (Broekkamp et al., 1986; Molewijk et al., 1995; Teixeira et al., 2000), and clonidine (De Vry et al., 1993; Molewijk et al., 1995; Olsen et al., 2002). If the chick separation stress paradigm is a valid screen for traditional anxiolytics, then these compounds should demonstrate anxiolytic-like activity

* Corresponding author. Department of Psychology, University of Mississippi, Oxford, MS 38677, USA. Tel.: +1-662-915-7728; fax: +1-662-915-5398.
E-mail address: pysufka@olemiss.edu (K.J. Sufka).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.10.019

in the test. Furthermore, to insure that the paradigm is insensitive to false positives, five compounds that have shown either no activity or anxiogenic effects in other paradigms were also tested (i.e., negative controls), including amphetamine (Vogel et al., 1971; Howard et al., 1982; Salt and Taberner, 1984; Pellow et al., 1985; Lecci et al., 1990; Shimada et al., 1995; Hascoet and Bourin, 1998; Olsen et al., 2002), scopolamine (Vogel et al., 1971; Shimada et al., 1995; Smythe et al., 1996), caffeine (Pellow et al., 1985; Baldwin et al., 1989; Shimada et al., 1995; Bhattacharya et al., 1997; Hascoet and Bourin, 1998), chlorpromazine (Crawley, 1981; Lecci et al., 1990), and haloperidol (Russell et al., 1987; Rodina et al., 1993; Sanchez, 2003). If the chick separation stress paradigm is similarly insensitive to false positives, then these compounds should show no activity or anxiogenic-like activity in the test. Thus, the purpose of this research is to further validate the chick separation stress paradigm as an anxiolytic screen.

2. Materials and methods

2.1. Subjects

Cockerels (*Gallus gallus*; strain W36; Cal-Maine Foods, Mendenhall, MS, USA) were obtained 1-day posthatch and were housed in stainless steel cages (34 × 57 × 40 cm) at a population density of 12–13 chicks per cage. Food (Purina Start and Grow, St. Louis, MO) and water were available ad libitum through 1-qt gravity-fed feeders and waterers. Room temperature was maintained at 29 ± 1 °C, and overhead fluorescent illumination was maintained on a 12-h light–dark cycle. Daily maintenance was conducted during the first quarter of the light cycle.

2.2. Apparatus

The six-unit test apparatus contained Plexiglas viewing chambers (25 × 25 × 22 cm) situated in sound-attenuating enclosures. Each unit was illuminated by a 25-W light bulb and ventilated by an 8-cm-diameter rotary fan (Commonwealth Model FP-108AX S1). Miniature video cameras (SuperCircuit Model PC47MC) allowed for animal observation during tests. Distress vocalizations were recorded by microphones (Lafayette Instruments Model 3-675-001) mounted at the ceiling of the Plexiglas chamber and connected to digital sound-activating relays (Lafayette Instruments Model 63040A; settings: 75% sensitivity and 0.10-s delay) that triggered electromechanical counters (Lafayette Instruments Model 58004).

2.3. Procedure

Experiments were conducted at 7 days posthatch. Groups formed a single factorial design with a hanging control that included two vehicle-control groups in which chicks were tested in isolation, with or without two mirrors (20 × 20 cm) positioned along the outside of the Plexiglas side walls, and four drug dose conditions tested under the no-mirror condition (except for three doses of caffeine). The positive drug probes were meprobamate (15, 30, 60, or 120 mg/kg), pentobarbital (2.5, 5.0, 10.0, or 20.0 mg/kg), chlordiazepoxide (2.5, 5.0, 10.0, or 15.0 mg/kg), buspirone (2.5, 5.0, 7.5, or 10.0 mg/kg), imipramine (1, 3, 10, or 15 mg/kg), and clonidine (0.1, 0.15, 0.2, or 0.25 mg/kg). The negative drug probes were amphetamine (0.5, 1.0, 2.0, or 4.0 mg/kg), scopolamine (0.2, 0.4, 0.8, or 1.6 mg/kg), caffeine (5, 10, or 20 mg/kg), chlorpromazine (1, 3, 10, or 30 mg/kg), and haloperidol (0.03, 0.1, 0.3, or 1.0 mg/kg). The vehicle for all experiments was 0.9% physiological saline, except for the meprobamate experiment, which was 100% propylene glycol. Drug doses were based on previous literature in rodent models of anxiety and pilot studies in this laboratory.

Vehicle and drug injections were administered intramuscularly 15 min before tests. The stress manipulation involved placing a chick in the observation chamber either in a mirror (low-stress) or no-mirror (high-stress) condition for a 180-s test period. Dependent measures collected during the test session were (1) distress vocalizations and (2) sleep onset latency, defined as the latency to adopt a posture in which the chick's head is drooping and its eyes are closed. Animals were returned to their home cage following tests. These procedures were approved by the University of Mississippi IACUC (Protocol No. 3-010) and were conducted in accordance with the principles of laboratory animal care as detailed in the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Data were analyzed using *t* tests and analysis of variance (ANOVA). Post hoc analyses were conducted using Fisher's LSD. Percent anxiolytic effect scores were derived from the following formula using the mean distress vocalizations for each group under the no-mirror condition: percent anxiolytic effect=[1 − (drug probe/vehicle)] × 100. Percent sedative effect scores were derived from the following formula: percent sedative effect=[1 − (mean sleep onset latency/180)] × 100.

3. Results

3.1. Positive drug probes

The descriptive statistics for the positive drug probe experiments on distress vocalizations are summarized in Table 1. For all positive drug probe experiments, vehicle/no-mirror chicks emitted a greater number of distress vocalizations than vehicle/mirror chicks [$ts(28–31) = 3.96–9.94$, $Ps \leq .001$], an effect that was dose-dependently attenuated by all positive drug probes except buspirone; ANOVAs: meprobamate [$F(4,74) = 6.64$, $P < .0005$], pentobarbital [$F(4,74) = 38.07$, $P < .0001$], chlordiazepoxide [$F(4,74) = 28.86$, $P < .0001$], imipramine [$F(4,72) = 3.34$,

Table 1
Effect of positive drug probes on mean distress vocalizations and mean sleep onset latency

	n	DVoc, mean (S.E.M.)	SOL, mean (S.E.M.)
Meprobamate			
Vehicle/mirror	15	91.40 (22.89)	170.33 (9.67)
Vehicle/no-mirror	18	259.50 (10.49)*	180.00 (0.00)
15 mg/kg	15	236.13 (13.35)	180.00 (0.00)
30 mg/kg	15	267.20 (13.10)	180.00 (0.00)
60 mg/kg	15	241.40 (12.13)	180.00 (0.00)
120 mg/kg	16	149.69 (32.78)**	136.06 (17.41)†
Pentobarbital			
Vehicle/mirror	16	14.69 (4.24)	180.00 (0.00)
Vehicle/no-mirror	16	217.81 (19.99)*	180.00 (0.00)
2.5 mg/kg	16	194.50 (16.96)	171.00 (9.00)
5.0 mg/kg	16	191.63 (19.13)	162.50 (10.17)
10.0 mg/kg	15	34.47 (17.85)**	62.40 (19.47)†
20.0 mg/kg	16	0.00 (0.00)**	1.75 (1.45)†
Chlordiazepoxide			
Vehicle/mirror	16	35.19 (14.26)	180.00 (0.00)
Vehicle/no-mirror	16	187.13 (19.82)*	171.88 (8.13)
2.5 mg/kg	16	220.19 (17.36)	180.00 (0.00)
5.0 mg/kg	16	134.31 (25.21)**	132.81 (15.99)†
10.0 mg/kg	15	30.20 (14.55)**	59.93 (17.41)†
15.0 mg/kg	16	3.88 (3.50)**	51.88 (16.16)†
Buspirone			
Vehicle/mirror	15	128.20 (16.96)	180.00 (0.00)
Vehicle/no-mirror	15	263.20 (29.56)*	180.00 (0.00)
2.5 mg/kg	14	265.57 (22.80)	180.00 (0.00)
5.0 mg/kg	16	303.31 (23.71)	180.00 (0.00)
7.5 mg/kg	15	321.47 (26.21)	180.00 (0.00)
10.0 mg/kg	15	326.87 (10.96)	180.00 (0.00)
Imipramine			
Vehicle/mirror	15	27.93 (9.88)	168.00 (12.00)
Vehicle/no-mirror	16	156.00 (16.46)*	152.00 (15.25)
1 mg/kg	16	110.88 (23.10)	180.00 (0.00)‡
3 mg/kg	17	111.53 (18.00)	180.00 (0.00)‡
10 mg/kg	17	89.35 (22.80)**	180.00 (0.00)‡
15 mg/kg	11	47.09 (16.53)**	147.27 (21.95)
Clonidine			
Vehicle/mirror	15	42.67 (18.00)	180.00 (0.00)
Vehicle/no-mirror	15	186.93 (29.11)*	180.00 (0.00)
0.10 mg/kg	15	140.47 (32.16)	148.60 (14.12)
0.15 mg/kg	15	0.00 (0.00)**	60.33 (14.12)†
0.20 mg/kg	14	2.14 (2.14)**	84.50 (18.53)†
0.25 mg/kg	15	1.07 (0.93)**	73.13 (15.86)†

DVoc = distress vocalizations; SOL = sleep onset latency.
* Significant stress effect, $P < .05$.
** Significant attenuation of the stress effect, $P < .05$.
† Significant sedative effect, $P < .05$.
‡ Indicates a significant increase in sleep onset latency scores. $P < .05$.

$P < .05$], and clonidine [$F(4,69) = 21.26$, $P < .0001$]. Post hoc analyses revealed a significant attenuation of distress vocalizations for chicks that received meprobamate (120 mg/kg), pentobarbital (10, 20 mg/kg), chlordiazepoxide (5, 10, 15 mg/kg), imipramine (10, 15 mg/kg), or clonidine (0.15, 0.2, 0.25 mg/kg) (Ps < .05).

The descriptive statistics for the positive drug probe experiments on sleep onset latency are summarized in Table 1. For all positive drug probe experiments, the vehicle did not affect the sleep onset latency scores for chicks tested in the mirror or no-mirror condition, while meprobamate, pentobarbital, chlordiazepoxide, and clonidine appeared to dose-dependently attenuate these scores; ANOVAs: meprobamate [$F(4,74) = 6.26$, $P < .0005$], pentobarbital [$F(4,74) = 59.59$, $P < .0001$], chlordiazepoxide [$F(4,74) = 21.06$, $P < .0001$], and clonidine [$F(4,69) = 14.07$, $P < .0001$]. Post hoc analyses revealed a significant attenuation of sleep onset latency scores for chicks that received meprobamate (120 mg/kg), pentobarbital (10, 20 mg/kg), chlordiazepoxide (5, 10, 15 mg/kg),

Table 2
Effect of negative drug probes on mean distress vocalizations and mean sleep onset latency

	n	DVoc, mean (S.E.M.)	SOL, mean (S.E.M.)
Amphetamine			
Vehicle/mirror	14	122.93 (31.30)	180.00 (0.00)
Vehicle/no-mirror	17	231.77 (18.67)*	169.41 (10.59)
0.5 mg/kg	14	260.50 (22.75)	174.50 (5.50)
1.0 mg/kg	15	235.47 (18.93)	180.00 (0.00)
2.0 mg/kg	14	241.86 (11.34)	180.00 (0.00)
4.0 mg/kg	16	25.19 (15.28)**	102.94 (21.18)†
Scopolamine			
Vehicle/mirror	16	37.13 (14.65)	180.00 (0.00)
Vehicle/no-mirror	16	181.75 (21.56)*	180.00 (0.00)
0.2 mg/kg	15	199.53 (16.84)	180.00 (0.00)
0.4 mg/kg	16	162.31 (17.80)	180.00 (0.00)
0.8 mg/kg	16	188.19 (20.51)	180.00 (0.00)
1.6 mg/kg	16	189.00 (22.72)	180.00 (0.00)
Caffeine			
Vehicle/mirror	17	63.06 (21.13)	169.41 (10.59)
Vehicle/no-mirror	17	213.88 (16.72)*	160.00 (13.70)
5 mg/kg	17	200.00 (16.70)	180.00 (0.00)
10 mg/kg	16	169.31 (26.67)	177.50 (2.50)
20 mg/kg	16	225.13 (25.24)	180.00 (0.00)
Chlorpromazine			
Vehicle/mirror	14	90.93 (26.60)	180.00 (0.00)
Vehicle/no-mirror	14	277.14 (18.30)*	180.00 (0.00)
1 mg/kg	14	258.71 (17.74)	180.00 (0.00)
3 mg/kg	13	263.92 (17.10)	180.00 (0.00)
10 mg/kg	14	214.43 (20.34)	180.00 (0.00)
30 mg/kg	14	225.00 (22.11)	180.00 (0.00)
Haloperidol			
Vehicle/mirror	16	43.25 (17.16)	180.00 (0.00)
Vehicle/no-mirror	16	241.06 (17.99)*	180.00 (0.00)
0.03 mg/kg	16	275.06 (11.33)	180.00 (0.00)
0.10 mg/kg	16	217.94 (21.45)	180.00 (0.00)
0.30 mg/kg	15	182.00 (29.87)	180.00 (0.00)
1.00 mg/kg	16	218.56 (21.20)	173.69 (6.31)

DVoc = distress vocalizations; SOL = sleep onset latency.
* Significant stress effect, $P < .05$.
** Significant attenuation of the stress effect, $P < .05$.
† Indicates a significant sedative effect. $P < .05$.

and clonidine (0.15, 0.2, 0.25 mg/kg) ($Ps < .05$). Although the ANOVA for the imipramine experiment was significant [$F(4,72) = 2.62$, $P < .05$], this effect was due to a significant increase in sleep onset latency scores for the 1, 3, and 10 mg/kg groups ($Ps < .05$). However, this significant imipramine effect is best accounted for by the somewhat lower sleep onset latency mean for the vehicle/no-mirror group.

3.2. Negative drug probes

The descriptive statistics for the negative drug probe experiments on distress vocalizations are summarized in Table 2. For all negative drug probe experiments, vehicle/no-mirror chicks emitted a greater number of distress vocalizations than vehicle/mirror chicks [$ts(26-32) = 3.12-7.96$, $Ps < .005$]. ANOVAs across these drug probes revealed a significant effect for the amphetamine and haloperidol experiments: amphetamine [$F(4,71) = 30.97$, $P < .0001$] and haloperidol [$F(4,74) = 2.63$, $P < .05$]. Post hoc analyses revealed a significant attenuation of distress vocalizations for chicks that received the 4-mg/kg dose of amphetamine ($P < .0001$) but no significant differences among the relevant haloperidol comparisons (i.e., drug dose vs. vehicle).

The descriptive statistics for the negative drug probe experiments on sleep onset latency are summarized in Table 2. For all negative drug probe experiments, the vehicle did not affect the sleep onset latency scores for chicks tested in the mirror or no-mirror condition, while only the 4-mg/kg amphetamine dose appeared to attenuate this measure; ANOVA: amphetamine [$F(4,71) = 8.42$, $P < .0001$]. Post hoc analyses revealed a significant attenuation of sleep onset latency scores for chicks that received the 4-mg/kg dose of amphetamine ($P < .0001$).

4. Discussion

The purpose of this study was to further validate the chick separation stress paradigm as a screen for anxiolytic compounds by extending its generalizability to nonbenzodiazepine anxiolytic compounds and by ensuring that the paradigm is not susceptible to false positives. In the positive drug probes experiment, the following compounds were evaluated in the paradigm: meprobamate, pentobarbital, chlordiazepoxide, buspirone, imipramine, and clonidine. In the negative drug probes experiment, the following compounds were evaluated in the paradigm: amphetamine, scopolamine, caffeine, chlorpromazine, and haloperidol. The two dependent measures were distress vocalizations as an index of separation stress and sleep onset latency to index sedation.

In all experiments, separation stress produced a stress effect as evidenced by increased vocalizations in chicks in the vehicle/no-mirror condition compared to chicks in the vehicle/mirror condition, an effect consistent with previous research (Feltenstein et al., 2002, 2003b). The separation stress effect was attenuated in a dose-dependent manner by all of the positive drug probes, with the exception of buspirone. The finding that clonidine, meprobamate, chlordiazepoxide, pentobarbital, and imipramine attenuate separation stress is consistent with previous studies using rodent models of anxiety (for reviews, see Green and Hodges, 1991; Stephens and Andrews, 1991; Borsini et al., 2002). It is not surprising that buspirone did not possess an anxiolytic-like effect in this paradigm. Previous studies have shown that chronic administration of buspirone is needed to induce an anxiolytic effect (Stephens and Andrews, 1991), an effect that is likely mediated by the desensitization of the 5-HT1A autoreceptor (Hjorth and Auerbach, 1996).

In all experiments, separation stress did not affect sleep onset latency in chicks in either vehicle group and this finding is consistent with previous research demonstrating an absence of a stress effect on a measure of sedation similar to sleep onset latency (Feltenstein et al., 2002, 2003b). Sleep onset latency was significantly affected in a dose-dependent manner by all of the positive drug probes, with the exception of buspirone and imipramine. The sedative effects of chlordiazepoxide (Watson and Sufka, 1996; Watson et al., 1999), clonidine (Cavero and Roach, 1978), meprobamate (Berger, 1954; Delong et al., 1985; Lambdin et al., 2002), and pentobarbital (Harvey, 1980; Lambdin et al., 2002) in chicks are consistent with previous studies. However, it is interesting to note that for those drugs that induced both anxiolytic and sedative effects, the anxiolytic effect was always greater than the sedative effect (see Table 3).

Table 3
Percent anxiolytic effect compared to the percent sedative effect

	Dose 1	Dose 2	Dose 3	Dose 4
Meprobamate				
DVoc	9.01	− 2.97	6.97	42.32**
SOL	0.00	0.00	0.00	24.41†
Pentobarbital				
DVoc	10.70	12.02	84.17**	100.00**
SOL	5.00	9.72	65.33†	99.03†
Chlordiazepoxide				
DVoc	− 17.67	28.23**	83.86**	97.93**
SOL	0.00	26.22†	66.70†	71.18†
Buspirone				
DVoc	− 0.91	− 15.24	− 22.14	− 24.19
SOL	0.00	0.00	0.00	0.00
Imipramine				
DVoc	28.92	28.51	42.72**	69.81**
SOL	− 18.42‡	− 18.42‡	− 18.42‡	3.11
Clonidine				
DVoc	24.85	100.00**	98.86**	99.43**
SOL	17.44	66.48†	53.06†	59.37†

DVoc = distress vocalizations; SOL = sleep onset latency.
** Significant attenuation of the stress effect, $P < .05$.
† Significant sedative effect, $P < .05$.
‡ Significant increase in sleep onset latency scores, $P < .05$.

In all negative drug probe experiments, neither distress vocalizations nor sleep onset latency was affected by any dose of the probes, with the exception of the highest dose of amphetamine. While 4 mg amphetamine did affect sleep onset latency scores, this effect was not due to sedation, but rather marked signs of stereotypy. A number of animals in this amphetamine group adopted a posture that met the operational criteria for scoring a sleep onset latency response. However, these chicks also exhibited a feature not found in other chicks and that was repetitive head movements, a stereotyped behavior commonly seen in other animals. Using the Wolgin (1995) stereotypy behavior rating scale, where 0 represents the *animal being stationary and immobile* and 5 represents *oral stereotypy*, comments written during testing indicated several chicks in this amphetamine group exhibited a 4 rating by displaying focused repetitive head movements. This stereotyped behavior was easily distinguished from general sedation and suggests the need to include such an exclusionary measure into the procedure for drugs possessing stimulant properties.

Collectively, these experiments support the notion that the chick separation stress paradigm can be used as a high-throughput anxiolytic screen (Willner, 1991). Although other animal models exist for screening anxiolytic drugs (Green and Hodges, 1991), the chick separation stress paradigm possesses many practical advantages. It is extremely cost-effective in that chicks are inexpensive to purchase and maintain and they require small quantities of drug in the screening process. The procedure also measures a species-typical response rather than time- and labor-intensive conditioned behaviors. While the current study demonstrates the paradigm is sensitive to a wide range of anxiolytic compounds and insensitive to a wide range of negative drug probes, it, like other rodent-based models, was insensitive to acute administration of buspirone. To understand this limitation, current studies are investigating whether chronic administration of buspirone is necessary to produce anxiolytic activity in this paradigm.

Acknowledgements

The authors thank E. Byrd, C. Crenshaw, C. Decker, J. Desai, C. Marks, M. McGehee, B. Schneider, J. Stewart, and A. Taylor for their assistance in data collection, as well as Cal-Maine Foods for their generous donation of research animals.

References

Baldwin HA, Johnston AL, File SE. Antagonistic effects of caffeine and yohimbine in animal tests of anxiety. Eur J Pharmacol. 1989;159:211–5.

Berger FM. The pharmacological properties of 2-methyl-2-*n*-propyl-1,3 propanediol dicarbamate (Miltown), a new interneuronal blocking agent. J. Pharmacol. Exp. Ther. 1954;112:413–23.

Bhattacharya SK, Satyan KS, Chakrabarti A. Anxiogenic action of caffeine: an experimental study in rats. J. Psychopharmacol. 1997;11:219–24.

Borsini F, Podhorna J, Marazziti D. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology (Berl.) 2002;163:121–41.

Broekkamp CL, Rijk HW, Joly-Gelouin D, Lloyd KL. Major tranquilizers can be distinguished from minor tranquilizers on the basis of effects on marble burying and swim-induced grooming in mice. Eur J Pharmacol 1986;126:223–9.

Cavero I, Roach AG. The effects of prazosin on the clonidine induced hypotension and bradycardia in rats and sedation in chicks ([proceedings])Br J Pharmacol 1978;62:468P–9P.

Crawley JN. Neuropharmacologic specificity of a simple animal model for the behavioral actions using benzodiazepines. Pharmacol Biochem Behav 1981;15:695–9.

Delong RE, Phillis JW, Barraco RA. A possible role of endogenous adenosine in the sedative action of meprobamate. Eur J Pharmacol 1985; 118:359–62.

De Vry J, Benz U, Schreiber R, Traber J. Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. Eur J Pharmacol 1993;249:331–9.

Feltenstein MW, Ford NG, Freeman KB, Sufka KJ. Dissociation of stress behaviors in the chick social-separation-stress procedure. Physiol Behav 2002;75:675–9.

Feltenstein MW, Lambdin LC, Ganzera M, Dharmaratne HRW, Nanayakkara NPD, Khan IA, et al. Anxiolytic properties of *Piper methysticum* extract samples and fractions in the chick social-separation-stress procedure. Phytother Res 2003a;17:210–6.

Feltenstein MW, Lambdin LC, Webb HE, Warnick JE, Khan SI, Khan IA, et al. Corticosterone response in the chick separation-stress paradigm. Physiol Behav 2003b;78:489–93.

Geller I, Seifter J. The effects of meprobamate, barbiturates, D-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1960;1:482–92.

Graeff FG, Netto CF, Zangrossi Jr H. The elevated T-maze as an experimental model of anxiety. Neurosci Biobehav Rev 1998;23:237–46.

Green S, Hodges H. Animal models of anxiety. In: Willner P, editor. Behavioural models in psychopharmacology: theoretical, industrial and clinical perspectives. Cambridge (MA): Cambridge Univ. Press; 1991. p. 21–49.

Harvey SC. Hypnotics and sedatives. In: Gilman AG, Goodman LS, Gilman A, editors. The pharmacological basis of therapeutics. 6th ed. New York: Macmillan; 1980. p. 339–75.

Hascoet M, Bourin M. A new approach to the light/dark test procedure in mice. Pharmacol Biochem Behav 1998;60:645–53.

Hjorth S, Auerbach SB. 5-HT1A autoreceptors and the mode of action of selective serotonin reuptake inhibitors (SSRI). Behav Brain Res 1996;73:281–3.

Howard JL, Rohrbach KW, Pollard GT. Cumulative dose–effect curves in a conflict test with incremental shock. Psychopharmacology (Berl.) 1982;78:195–6.

King JA, Messenger T, Ferris CF. Seed finding in golden hamsters: a potential animal model for screening anxiolytic drugs. Neuropsychobiology 2002;45:150–5.

Lambdin LC, Guth AN, Feltenstein MW, Sufka KJ. Validation of the chick social-separation stress procedure as a screen for anxiolytic compounds. Soc.Neurosci Abstr 2002;571.20.

Lecci A, Borsini F, Volterra G, Meli A. Pharmacological validation of a novel animal model of anticipatory anxiety in mice. Psychopharmacology (Berl.) 1990;101:255–61.

McCloskey TC, Paul BK, Commissaris RL. Buspirone effects in an animal conflict procedure: comparison with diazepam and Phenobarbital. Pharmacol Biochem Behav 1987;27:171–5.

Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol 2003;70:83–244.

Molewijk HE, van der Poel AM, Mos J, van der Heyden JA, Olivier B. Conditioned ultrasonic distress vocalizations in adult male rats as a

behavioural paradigm for screening anti-panic drugs. Psychopharmacology (Berl.) 1995;117:32–40.

Olsen CK, Hogg S, Lapiz MD. Tonic immobility in guinea pigs: a behavioural response for detecting an anxiolytic-like effect? Behav Pharmacol 2002;13:261–9.

Panksepp J, Meeker R, Bean NJ. The neurochemical control of crying. Pharmacol Biochem Behav, 1980;12:437–43.

Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

Rodina VI, Krupina NA, Kryzhanovskii GN. A new natural model of elevated anxiety in rats. Biull Eksp Biol Med 1993;116:127–30.

Rowan MJ, Cullen WK, Moulton B. Buspirone impairment of performance of passive avoidance and spatial learning tasks in the rat. Psychopharmacology (Berl.) 1990;100:393–8.

Russell KH, Hagenmeyer-Houser SH, Sanberg PR. Haloperidol-induced emotional defecation: a possible model for neuroleptic anxiety syndrome. Psychopharmacology (Berl.) 1987;91:45–9.

Salt JS, Taberner PV. Differential effects of benzodiazepines and amphetamine on exploratory behaviour in weanling rats: an animal model for anxiolytic activity. Prog Neuropsychopharmacol 1984;8:163–9.

Sanchez C. Stress-induced vocalization in adult animals. A valid model of anxiety? Eur. J. Pharmacol. 2003;463:133–43.

Shimada T, Matsumoto K, Osanai M, Matsuda H, Terasawa K, Watanabe H. The modified light/dark transition test in mice: evaluation of classic and putative anxiolytic and anxiogenics drugs. Gen Pharmacol 1995;26:205–10.

Smith KK, Dharmaratne HRW, Feltenstein MW, Broom SL, Roach JT, Nanayakkara NPD, et al. Anxiolytic effects of kava extract and kavalactones in the chick social separation-stress paradigm. Psychopharmacology (Berl.) 2001;155:86–90.

Smythe JW, Murphy D, Bhatnagar S, Timothy C, Costall B. Muscarinic antagonists are anxiogenic in rats tested in the black–white box. Pharmacol. Biochem. Behav. 1996;54:57–63.

Stephens DN, Andrews JS. Screening for anxiolytic drugs. In: Willner P, editor. Behavioural models in psychopharmacology: theoretical, industrial and clinical perspectives. Cambridge (MA): Cambridge Univ. Press; 1991. p. 50–75.

Sufka KJ, Weed NC. Construct validation of behavioral indices of isolation stress and inflammatory nociception in young domestic fowl. Physiol Behav 1994;55:741–6.

Sufka KJ, Hughes RA, McCormick TM, Borland JL. Opiate effects on isolation stress in domestic fowl. Pharmacol Biochem Behav 1994;49:1011–5.

Sufka KJ, Roach JT, Chambliss WJ, Broom SL, Feltenstein MW, Wyandt CM, et al. Anxiolytic properties of botanical extracts in the chick social separation-stress procedure. Psychopharmacology (Berl.) 2001;153:219–24.

Teixeira RC, Zangrossi H, Graeff FG. Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. Pharmacol Biochem Behav 2000;65:571–6.

Vogel JR, Beer B, Clody DE. A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 1971;21:1–7.

Watson GS, Sufka KJ. Chlordiazepoxide reverses social-separation-induced distress vocalizations and analgesia in young domestic fowl. Exp Clin Psychopharmacol 1996;4:347–53.

Watson GS, Roach JT, Sufka KJ. Benzodiazepine receptor function in the chick social separation-stress procedure. Exp Clin Psychopharmacol 1999;7:83–9.

Willner P. Behavioural models in psychopharmacology. In: Willner P, editor. Behavioural models in psychopharmacology. Theoretical, industrial, and clinical perspectives. Cambridge (MA): Cambridge Univ. Press; 1991. p. 3–18.

Wolgin DL. Development and reversal of sensitization to amphetamine-induced hypophagia: role of temporal, pharmacological, and behavioral variables. Psychopharmacology 1995;117:49–54.