Pharmacology, Biochemistry and Behavior 92 (2009) 413–423

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of pretest manipulation on elevated plus-maze behavior in adolescent and adult male and female Sprague–Dawley rats

Tamara L. Doremus-Fitzwater, Elena I. Varlinskaya, Linda Patia Spear *

Center for Development and Behavioral Neuroscience, Department of Psychology, Binghamton University, Binghamton, New York 13902-6000, USA

ARTICLE INFO

Article history:
Received 28 June 2008
Received in revised form 9 December 2008
Accepted 13 January 2009
Available online 22 January 2009

Keywords:
Adolescence
Elevated plus-maze
Anxiety
Pretest manipulation
Rats
Age differences
Sex differences

ABSTRACT

The elevated plus-maze (EPM) is vulnerable to variations in pretest circumstances when testing adult rodents. Because of an increasing interest in adolescence, the present experiments examined the impact of pretest manipulations on anxiety levels in the EPM among adolescent and adult Sprague–Dawley rats of both sexes. In Exp. 1, animals removed from their home cage and immediately placed on the EPM were compared to rats tested following 30 min of social isolation, or following 30-min exposure to a novel context. These pretest manipulations only modestly decreased anxiety levels at both ages. In Exp. 2, more varied pretest conditions were examined: testing directly from the home cage; testing following 30 min of social isolation in a novel environment; or a large saline injection and rehousing 18 h prior to a 30-min period of social isolation in a novelty situation before testing. In adults, anxiety levels decreased linearly as pretest perturbation increased, whereas adolescents showed comparable levels of anxiety with both the moderate and large perturbations. As a result, observed age differences in anxiety differed as a function of pretest circumstances. Therefore, caution is urged when using the EPM for across-age comparisons of anxiolytic and anxiogenic effects of pharmacological or other manipulations.

1. Introduction

The elevated plus-maze (EPM) is a widely used model for the study of anxiety-like behavior in rodents. This plus-shaped apparatus is elevated from the floor and consists of two arms with walls and two arms that are open platforms. Since the maze presents a conflict of motivation to explore the novel yet risky open arms against the motivation to remain safe in the enclosed arms, the proportion of time an animal spends in the open versus closed arms is believed to provide an index of that animal's anxiety level (for reviews see Carobrez and Bertoglio, 2005; Wall and Messier, 2001).

Substantial prior research has focused on validating the EPM as a behavioral assay of anxiety in adult rodents. Pharmacological studies have documented that anxiolytic drugs (e.g. diazepam) effectively increase the proportion of time animals spend in the open relative to the closed arms, reflecting decreased anxiety in these animals (Pellow et al., 1985; Wilson et al., 2004). In contrast, anxiogenic drugs (e.g. pentylenetetrazol) have been shown to have the opposite effect, with animals increasing the amount of time spent in the closed arms and avoiding the open arms to a greater extent (Wada and Fukuda, 1991; Wallis and Lal, 1998). Moreover, drugs that should not impact anxiety levels, such as haloperidol, have been shown to decrease overall locomotor activity on the maze while not affecting percentage of open arm activity (Pellow et al., 1985). File and colleagues further validated the EPM in terms of the hormonal responses to fearful and anxiety-provoking situations. They found that adult animals had much greater corticosterone (CORT) response following confinement to open arms than to closed arms in the EPM (Pellow et al., 1985), with other researchers also demonstrating similar increases in CORT levels following open arm confinement (Degroot et al., 2004; McCormick et al., 2008). These increases in CORT have been taken as evidence of increased anxiety.

Within the field of psychology, there has been increasing emphasis on adolescence as a time of notable increases in expression of mental health disorders including schizophrenia, substance abuse and depression (see Kessler et al., 2005 for review). Ontogenetic alterations are also seen in the expression of specific anxiety disorders and in the efficacy of their treatment with different classes of anxiolytic drugs (Foa et al., 2005). Characterized by the transition from dependence to independence, this ontogenetic phase is associated with numerous conserved neural, behavioral and hormonal features that are evident not only in human adolescents, but in organisms undergoing this transition in other species as well (Spear, 2000). These across-species commonalities in basic neurochemical and hormonal characteristics of adolescence provide support for the judicious use of animal models of adolescence, particularly under circumstances where such research

* Corresponding author. Department of Psychology, Binghamton University PO Box 6000, State University of New York, Binghamton, NY 13902-6000, USA. Tel.: +1 607 777 2825; fax: +1 607 777 6418.
E-mail address: lspear@binghamton.edu (L.P. Spear).

0091-3057/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.pbb.2009.01.006

with human youths would be difficult or unethical. In the rat, adolescence has been conservatively defined as postnatal days (P) 28 to 42 (Spear, 2000), although the exact onset and end of adolescence is a gradual one. During this period, adolescent rats (like their human counterparts) show an increased prevalence of behaviors such as risk-taking, novelty seeking and peer-directed social behaviors (for reviews see Adriani et al., 2004; Spear, 2000). The expression of these behaviors, combined with the challenging transitions that adolescents face (e.g. emigration from the family unit and establishment of mature relationships with peers), has lead to the hypothesis that adolescence may be a relatively stressful phase characterized by heightened anxiety and greater stress reactivity than seen among younger or more mature individuals (e.g. Spear, 2000; Walker et al., 2001).

To answer the question as to whether adolescents are more anxious than adults, and to explore the potential efficacy of novel anxiolytics among adolescents, researchers have primarily turned to the EPM and other well-established anxiety models developed and validated for use in adult rodents. Using these models, findings regarding ontogenetic changes in basal anxiety levels have been quite inconsistent, with adolescents reported to exhibit more (Hascoet et al., 1999; Slawecki, 2005), less (Imhof et al., 1993) or similar levels of anxiety as adults (Slawecki and Roth, 2004; Varlinskaya and Spear, 2002, 2008). While some of these inconsistencies may be related to differences across anxiety models (e.g. the light-dark box versus the EPM), a comparison of the results obtained within studies using the EPM alone have still yielded notable inconsistencies. Several EPM studies have reported that younger animals are more anxious than older animals (e.g. Doremus et al., 2003), whereas others have reported the opposite (e.g. Andrade et al., 2003; Imhof et al., 1993), or no age differences (e.g. Hefner and Holmes, 2007; Walker et al., 2004). Even within our own laboratory, comparison of control non-drug-exposed animals across different studies sometimes have yielded disparate conclusions regarding age-related differences in anxiety-like behavior (Doremus-Fitzwater and Spear, 2007; Doremus-Fitzwater et al., 2006; Doremus et al., 2003).

If tests such as the EPM are to be used to probe anxiety-related behaviors during adolescence, they should be validated for use with adolescents. As part of this process, factor analyses of several EPM data sets collected within our laboratory revealed primary underlying components of EPM behavior that were similar in both adolescents and adults, with anxiety-like measures consistently loading on the first factor and activity on the second (Doremus et al., 2006). While these results support the conclusion that similar constructs are being measured in adolescents and adults in this test, other issues also need to be addressed. Of particular importance, there is substantial evidence that basal anxiety levels in adults are sensitive to manipulations occurring prior to EPM testing (for reviews see Carobrez and Bertoglio, 2005; Hogg, 1996; Rodgers and Dalvi, 1997). To give but a few examples, studies have shown that pretest perturbations such as handling (Schmitt and Hiemke, 1998), transportation on a cart (Morato and Brandao, 1996) and housing condition (Haller and Halasz, 1999; Schmitt and Hiemke, 1998) each alter EPM anxiety levels among adults. Furthermore, results from our lab have suggested that the susceptibility of anxiety levels to be affected by pretest manipulations may vary with age, with, for instance a 5-min holeboard exposure prior to the EPM test differentially impacting anxiety levels in adolescents compared to adults (Doremus-Fitzwater and Spear, 2007). Effects of ontogenetic differences in response to pretest manipulations have not been systematically investigated, however, despite potential implications of age differences in pretest lability for conclusions drawn in across-age studies of anxiety-related indices in the EPM. Therefore, the purpose of the present study was to systematically explore the influence of pretest perturbation on anxiety-like behavior indexed in the EPM in both adolescent and adult rats.

2. General methods

At all times, animals used in the current experiments were maintained and treated in accordance with the guidelines for animal care established by the National Institutes of Health (Institute of Laboratory Animal Resources, Commission on Life Sciences, 1996), using protocols approved by the Binghamton University Institutional Animal Care and Use Committee (IACUC). In order to reduce the impact of litter effects, no more than one male and one female animal from a given litter was placed into any particular experimental group in any of these experiments (Holson and Pearce, 1992).

2.1. Subjects

For both Exps. 1 and 2, Sprague–Dawley adolescent (P33–35) and adult (P70–75) rats were obtained from our in-house breeding facility. On the day after birth, P1, litters were culled to 8–10 pups, with 6 animals of one sex and 4 animals of the other being retained whenever possible. Offspring remained with their parents until weaning at P21, at which time they were pair-housed with a same-sex littermate. All animals were maintained in a temperature-controlled vivarium on a 14:10 h light:dark cycle (lights on at 0700 h), with ad libitum access to water and food (Purina rat chow, Lowell, MA). Given that both adolescents and adults were tested in these experiments, vaginal smears were not used to determine estrous cycle in the adult females.

2.2. Elevated plus-maze apparatus

The adult elevated plus-maze EPM consisted of two open arms, 48.3×12.7 cm, and two closed arms, $48.3 \times 12.7 \times 29.2$ cm. The adolescent EPM was proportionately sized based on crown-rump length and confirmed by gait width analysis, and consisted of 30×8.9 cm open arms and $30 \times 8.9 \times 20.3$ cm closed arms. Because our laboratory sometimes conducts EPM assessments following pharmacological manipulations that may disrupt balance/motor coordination, small plastic edges (.6 cm in height for adolescents and 1.3 cm for adults) were located along each side and end of the open arms to minimize the possibility of falling during testing (Fernandes and File, 1996). These edges ended 4.0 cm (adolescent) and 4.5 cm (adult) before the junctions of the open and closed arms to provide easy access below the plane of the maze, allowing for head-dipping behavior. Both mazes were elevated to a height of 50 cm. All sessions were conducted under dim light (3 lx), with no experimenter present in the room and a white noise generator (55 db) used to attenuate potentially distracting sounds during testing. Sessions were recorded by a camera mounted at a height of 147 cm, and were monitored and videotaped with equipment located in an adjacent room. Animals were tested sequentially, with no more than one animal present in the room for each session. After each animal, the apparatus was cleaned with a 3% hydrogen peroxide solution and dried before the next animal was tested.

2.3. Testing procedures

At the start of the EPM session, each subject was placed on the center platform facing a closed arm and its behavior on the maze videotaped for 5 min. Behavioral measures were later scored continuously from the videotapes by an experimenter blind to the experimental condition of each animal. Measures scored included time spent on the open and closed arms and number of entries into the open and closed arms. An arm entry was scored when all four paws were placed in the arm, whereas an exit was considered to have taken place when at least the two front paws were placed outside of the arm. Also measured were the number of protected and unprotected head dips; the former was defined as dipping the head

over the sides of the maze from within the center platform or a closed arm, whereas head dips were defined as unprotected when the same behavior occurred on an open arm. Similarly, stretched attend postures were recorded and also divided into protected and unprotected forms of these behaviors. The protected stretched attend posture was exhibited when the animal's two hind feet remained in a closed arm or the center platform while the animal elongated its head and shoulders forward, followed by subsequent retraction. Unprotected stretched attends were defined as the same behavior but when emitted while the animal was on an open arm.

Percentage of time spent on the open arms and percentage of open arm entries have repeatedly been shown to be reliable measures of anxiety on the EPM (Lal et al., 1991; Pellow et al., 1985). The measures of percent protected head dips and percent protected stretched attend postures have been suggested to be more ethologically relevant and more sensitive measures of anxiety, based on ethological analysis and pharmacological manipulations (Espejo, 1997; Rodgers and Cole, 1994; Rodgers and Dalvi, 1997). Closed arm entries have generally been considered an index of activity (Cruz et al., 1994; Rodgers and Dalvi, 1997). More recently, factor analyses have validated the EPM for use in both adolescent and adult rats, with similar primary and secondary behavioral components (i.e. anxiety and activity, respectively) emerging at both ages (Doremus et al., 2006).

2.4. Data analysis

Data were checked for outliers before analysis at each age, with a score >2.0 standard deviations from the mean of a particular experimental group being considered an outlier. Behaviors were compared across test conditions using between-group analysis of variance (ANOVA) procedures. Post-hoc analyses of significant main effects or interactions were assessed using Fischer's least significant difference (LSD) tests.

Experiment 1. Within our laboratory, we have accumulated data from several different studies using both adolescent and adult rats in the EPM. While data from these studies were primarily directed toward answering questions regarding age differences in sensitivity to psychopharmacological manipulations, data from control animals nevertheless provided the opportunity to assess age differences in

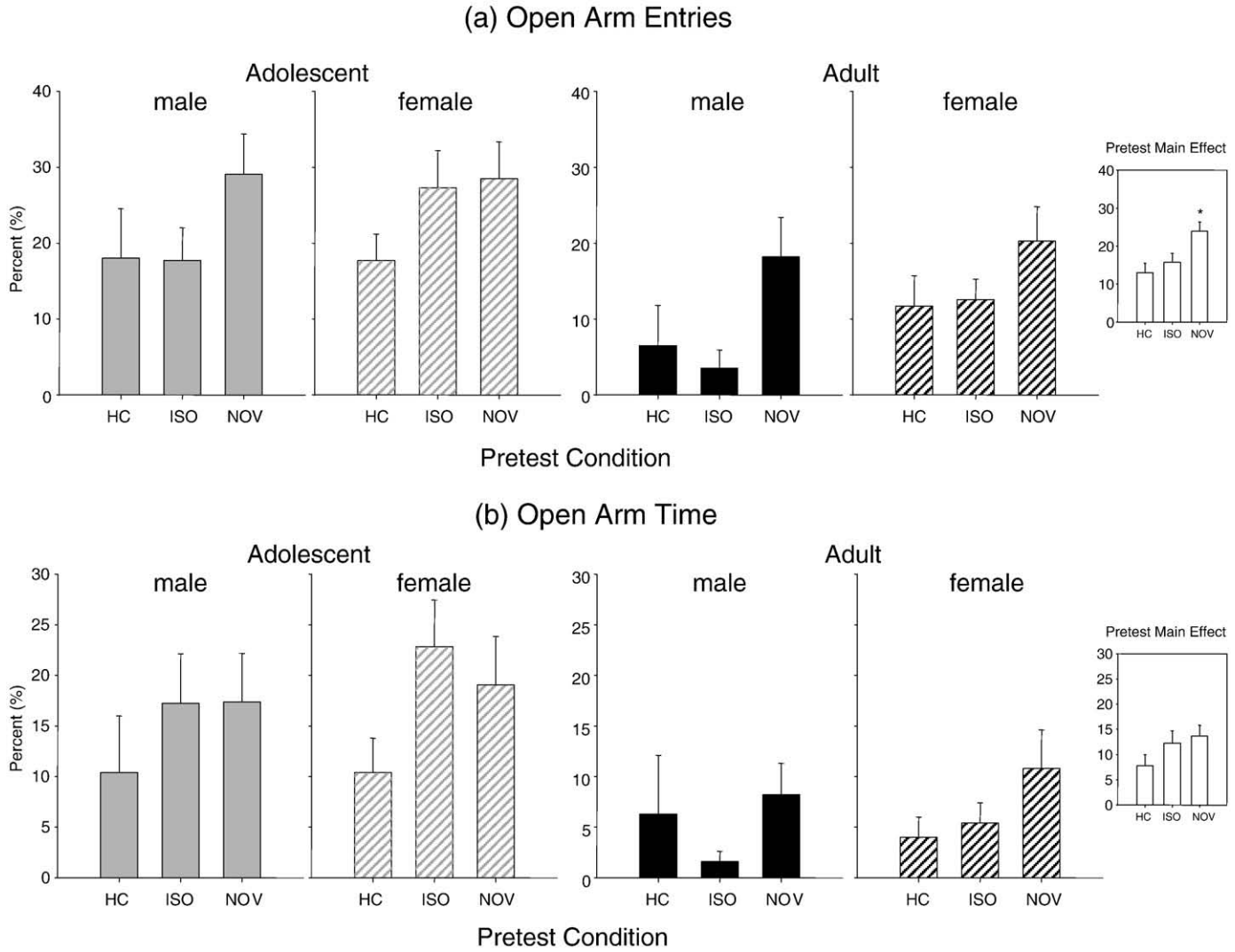

Fig. 1. Percent open arm entries (a) and percent open arm time (b) of adolescent and adult male (solid bars) and female (hatched bars) rats in the elevated plus-maze. Animals were tested either directly from the home cage (HC), following a 30-min period of social isolation in the home cage (ISO), or following a 30-min exposure to a novel cage with a familiar cagemate (NOV). Inserts are included for each behavior (collapsed across age and sex) in order to show the main effect of pretest condition, with asterisks denoting a significant difference from the home cage group. Eight animals were placed into each group, with the exception of 3 animals that fell from the maze, resulting in an *n* of 7 for the adolescent female novelty group, adult male isolation group, and adult female isolation group, with group sizes the same in Figs. 2 and 3.

anxiety. Unfortunately conclusions reached from comparisons of baseline data across experiments were not consistent, with adolescents sometimes found to be more anxious than adults (Doremus et al., 2003), or to exhibit similar anxiety levels as adults (Doremus-Fitzwater et al., 2006). Since the conditions under which our EPM testing was conducted were highly analogous across experiments, a likely contributor to these divergent ontogenetic patterns in anxiety behaviors was differences in the pretest manipulations used across these studies.

In an effort to examine possible age differences in the influence that pretest conditions may have on EPM behavior, Exp. 1 was designed to assess the impact of either pretest social isolation or pretest novelty on EPM behavior when compared to control animals tested directly from the home cage. These particular conditions were chosen, given that a period of social isolation in a novel environment prior to EPM testing is commonly used in our laboratory (Doremus-Fitzwater and Spear, 2007; Doremus et al., 2003; Wilmouth and Spear, 2006) and in other labs (e.g. Pellow et al., 1985; Prather et al., 1993), especially in pharmacological studies where animals are isolated for a period of time in a novel holding cage following drug injection to allow for drug absorption/distribution. The goal of this study was to determine whether novelty or social isolation prior to testing exerted a differential impact on subsequent EPM behavior, and whether these influences differed between adolescent and adult animals.

2.5. Methods

A total of 96 male and female adolescent and adult Sprague–Dawley rats were used across the 2 (age) × 2 (sex) × 3 (pretest: home cage vs. isolation vs. novelty) factorial design of the experiment ($n = 8$ per group).

Pretest conditions were as follows: (a) control animals tested directly from the home cage—these subjects were removed from the home cage containing their cagemate, immediately carried to an adjacent testing room, and placed on the EPM; (b) rats exposed to pretest social isolation—these animals were the cagemates of animals in the home cage condition. After their partners were removed from the cage for the EPM test, these animals were left alone in their home cage for 30 min prior to testing; (c) rats exposed to pretest novelty—novelty exposure was accomplished by removing a pair of rats from their home cage and placing them together in a novel holding cage for 30 min prior to testing. At the end of this pretest manipulation, one animal from the pair was immediately transferred from the holding cage to the EPM for testing.

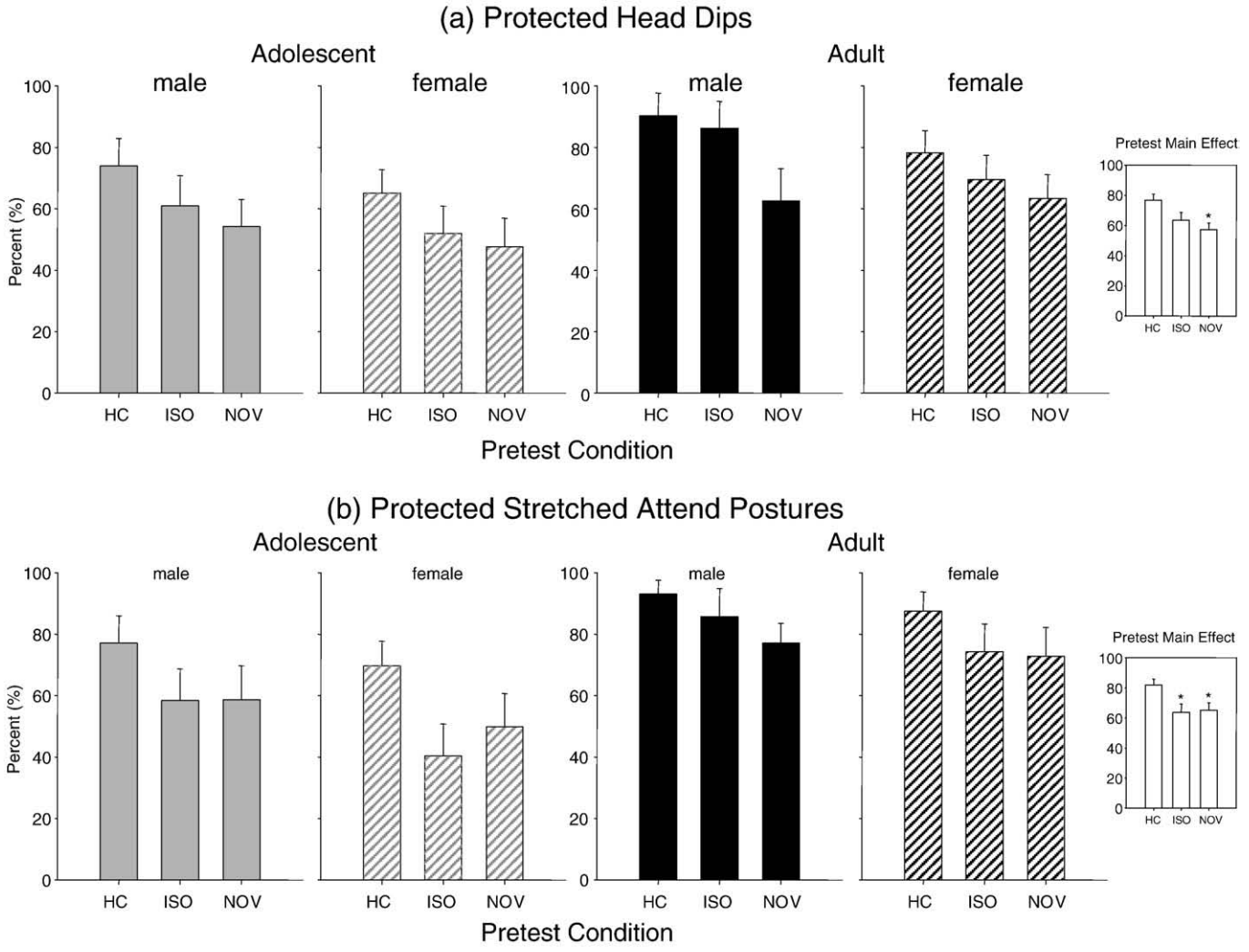

Fig. 2. Percent protected head dips (a) and percent protected stretched attend postures (b) of adolescent and adult male (solid bars) and female (hatched bars) rats in the elevated plus-maze. Animals were tested either directly from the home cage (HC), following a 30-min period of social isolation in the home cage (ISO), or following a 30-min exposure to a novel cage with a familiar cagemate (NOV). Inserts are included for each behavior (collapsed across age and sex) to emphasize the pretest main effect, with asterisks denoting a significant difference from the home cage group.

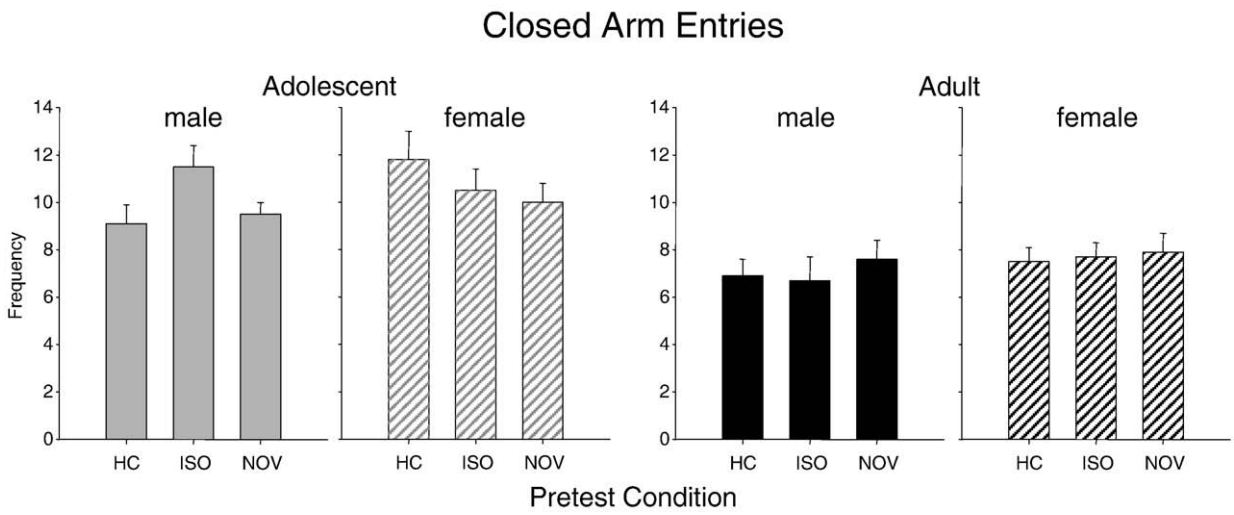

Fig. 3. Total number of closed arm entries exhibited by adolescent and adult male (solid bars) and female (hatched bars) rats in the elevated plus-maze. Animals were tested either directly from the home cage (HC), following a 30-min period of social isolation in the home cage (ISO), or following a 30-min exposure to a novel cage with a familiar cagemate (NOV).

2.6. Results

Three animals fell from the maze during testing and were not replaced: one adolescent female from the novelty condition, and one adult male and one adult female, both from the social isolation condition. In this data set, no outliers were present.

2.7. Anxiety

2.7.1. Pretest effects

When anxiety was measured via percent open arm entries (see Fig. 1a), animals that were exposed to pretest novelty but not isolation exhibited a significant reduction in anxiety levels [pretest main effect: $F(2,81)=5.85$, $p=.0042$], an effect that did not interact with age or sex. Neither pretest condition, however, significantly altered anxiety when indexed as percentage of time spent on the open arms (see Fig. 1b). In the analysis of protected head dipping behavior (Fig. 2a), pretest novelty again decreased anxiety levels [pretest main effect: $F(2,80)=5.44$, $p=.0061$] in adolescents and adults of both sexes, with pretest social isolation having no significant effects for this measure. Finally, when anxiety-like behavior was assessed as percentage of protected stretched attend postures (Fig. 2b), both pretest novelty and pretest isolation resulted in reduced anxiety levels [pretest main effect: $F(2,81)=5.08$, $p\leq.0084$], regardless of age or sex.

2.7.2. Age and sex effects

Adolescents were generally found to be significantly less anxious than adults [age main effect for percent open arm entries: $F(1,81)=16.38$, $p=.0001$; for percent open arm time: $F(1,81)=17.80$, $p\leq.0001$; for percent protected head dips: $F(1,80)=10.25$, $p=.002$; for percent protected stretched attend postures: $F(1,81)=19.56$, $p\leq.0001$], with no significant effects of sex observed in these analyses.

2.8. Activity

Assessment of activity, indexed via closed arm entries (Fig. 3), showed no significant effects of either pretest manipulation or sex.

Table 1
An across-study comparison of elevated plus-maze anxiety in adolescent and adult animals.

	Pretest condition[a]					
	(1)	(2)	(3)	(4)	(5)	(6)
%OAE[b] (↑ with ↓ anxiety)						
Adol	17.9 ± 3.6	22.5 ± 3.4	28.7 ± 3.5	19.7 ± 5.6	28.2 ± 3.9	23.6 ± 6.0
Adult[c]	*9.1 ± 3.3*	*8.0 ± 2.1*	*19.2 ± 3.3*	*22.7 ± 3.8*	*37.6 ± 4.8*	*36.5 ± 2.7*
%OAT (↑ with ↓ anxiety)						
Adol	10.4 ± 3.2	20.0 ± 3.3	18.2 ± 3.3	11.0 ± 3.3	18.7 ± 3.7	13.5 ± 5.1
Adult	*5.1 ± 3.0*	*3.5 ± 1.2*	*9.5 ± 2.4*	*17.2 ± 5.0*	*22.8 ± 5.1*	*25.9 ± 4.0*
%PHD (↓ with ↓ anxiety)						
Adol	73.4 ± 5.9	49.3 ± 7.5	54.6 ± 7.5	68.2 ± 8.6	55.1 ± 5.7	62.3 ± 9.9
Adult	*90.3 ± 3.8*	*80.0 ± 6.4*	*75.0 ± 5.6*	*61.0 ± 7.5*	*37.6 ± 10.9*	*44.5 ± 6.0*
%PSAP (↓ with ↓ anxiety)						
Adol	69.6 ± 5.8	56.4 ± 6.5	51.1 ± 6.2	69.1 ± 10.9	50.9 ± 9.4	62.1 ± 10.6
Adult	*84.2 ± 5.2*	*78.8 ± 5.9*	*63.0 ± 6.3*	*64.7 ± 10.7*	*34.3 ± 14.1*	*40.9 ± 11.7*

[a] Pretest conditions are presented from lower to higher degrees of pretest perturbation and originate from 3 different experimental studies. Conditions (1), (2) and (3) represent the three pretest conditions from Exp. 1 of the current series and were (1) tested directly from the home cage; (2) tested after 30 min of social isolation in the home cage; (3) tested following 30-min exposure to a novel holding cage. Since both male and female data were available for conditions (1), (2) and (3), these data are shown collapsed across sex. Pretest condition (4) was from an experiment testing acute anxiolytic drug actions in males and includes controls animals that were given a small vehicle injection followed by 30 min of social isolation in a novel environment (Doremus-Fitzwater et al., 2006). Male control animals from a study examining ethanol hangover (Doremus et al., 2003) comprised pretest conditions (5) and (6), with both of these groups of animals given a large saline injection and then rehoused 18 h prior to test. Group (5) animals received an additional period of social isolation plus novelty 30 min prior to test, whereas group (6) were stressed in a restraint tube for the 30-min pretest interval.

[b] Anxiety-like behavior was indexed via percent open arm entries (%OAE), percent open arm time (%OAT), percent protected head dips (%PHD) and percent protected stretched attend postures (%PSAP) following a variety of pretest manipulations in both adolescent (Adol) and adult rats.

[c] Note that in adults (italicized), anxiety-like behavior tended to decline with increases in pretest perturbations, whereas adolescents did not exhibit the same pattern of behavior in relation to pretest perturbation levels.

Adolescents did, however, exhibit significantly more closed arm entries than adults [main effect age: $F(1,81) = 40.27$, $p \leq .0001$].

Experiment 2. Taken together, the effects of the two different pretest manipulations examined in Exp. 1 were not striking, with novelty exposure seeming to have a somewhat greater, albeit still modest, influence on EPM anxiety levels than pretest isolation. Although these pretest effects did not differ with age, notable age differences in anxiety levels were evident, with adolescents being significantly less anxious than adults for all four of the anxiety measures that were assessed. This age difference in anxiety contrasts with earlier EPM results obtained from our laboratory where adolescents were found to be more (Doremus et al., 2003) or comparably anxious relative to adults (Doremus-Fitzwater and Spear, 2007; Doremus-Fitzwater et al., 2006). The pretest circumstances of these earlier studies, however, employed considerably more pretest manipulation than was used here.

In order to assess the impact that these more extreme pretest manipulations might have had (e.g. Doremus-Fitzwater and Spear, 2007; Doremus-Fitzwater et al., 2006) relative to the milder pretest perturbations used in Exp. 1, behavioral results from three different plus-maze experiments are presented together for comparison purposes (see Table 1). These data are shown (from left to right) from mild (lower numbers) to more extreme (higher numbers) levels of pretest perturbation. The pretest conditions used in Exp. 1 characterized the mild pretest perturbations shown in Table 1, with home cage animals as condition (1); pretest isolation as condition (2); pretest novelty as condition (3). Data representing a moderate level of pretest manipulation [condition (4) in Table 1] were obtained from male control animals in a study examining the acute effects of diazepam administration in the EPM (Doremus-Fitzwater et al., 2006). Male controls in that study were given a vehicle solution [Tween 80 (0.1% v/v) and cremaphor (2% v/v) in water at 2 ml/kg] and then socially isolated in a novel environment for 30 min prior to EPM testing. Finally, data from animals tested under more extreme pretest conditions were obtained from control male rats in a study investigating the anxiogenic effects of acute ethanol hangover (Doremus et al., 2003). These control animals were from one of two pretest conditions: (5) animals injected with saline (0.9% w/v at a volume of 2.52% of body weight) and re-housed with their partner overnight, with an additional 30-min period of pretest isolation in a novelty context given 18-h after injection and immediately prior to the EPM test; or (6) control animals treated identically except that they were subjected to 30 min of restraint stress immediately pretest (rather than the 30-min period of pretest isolation and novelty given to the former group).

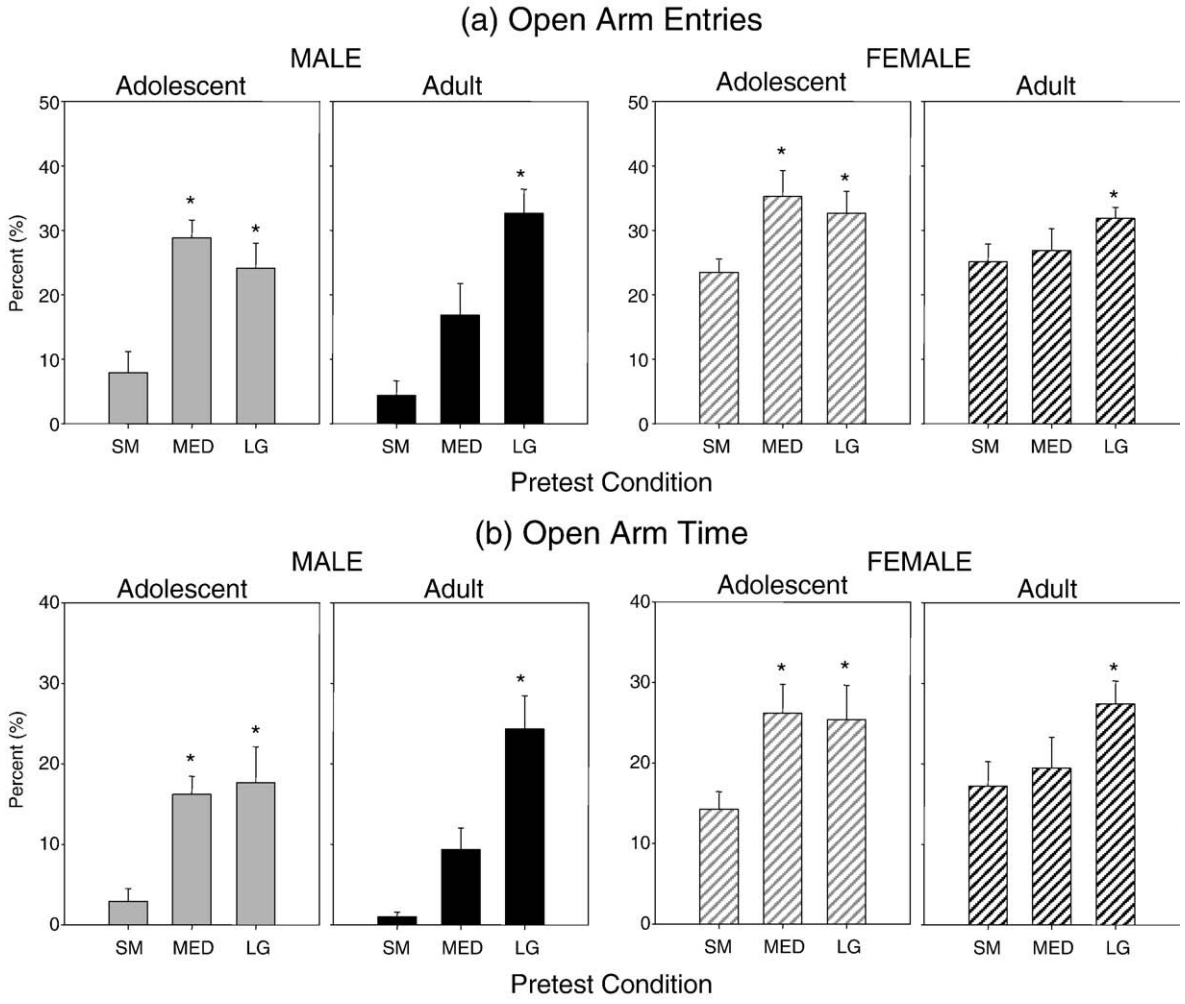

Fig. 4. Percent open arm entries (a) and percent open arm time (b) of adolescent and adult male (solid bars) and female (hatched bars) rats in the elevated plus-maze. Animals were tested either directly from the home cage (SM), following a 30-min period of social isolation in a novel environment (MED), or following a 30-min period of social isolation with novelty, preceded by rehousing and a large saline injection 18 h prior to testing (LG). Asterisks denote a significant difference from the home cage control group and are meant to emphasize the significant age x pretest interaction (i.e. when data were collapsed across sex). Group sizes were the same for Figs. 4–6 and were as follows: adolescent male small ($n = 9$) medium ($n = 9$); large ($n = 10$); adolescent female small ($n = 10$); medium ($n = 9$); large ($n = 9$); adult male small ($n = 8$); medium ($n = 10$); large ($n = 9$); adult female small ($n = 10$); medium ($n = 9$); large ($n = 8$).

When inspecting these data across experiments, adults (and to some extent, perhaps adolescents) appeared generally most anxious when tested directly from the home cage. Anxiety levels of adolescents varied only modestly across conditions, with no consistent pattern of anxiolysis relative to the degree of perturbation evident in this age group. Adults, in contrast, appeared to show a more consistent pattern of change across pretest conditions, with apparent declines in anxiety at progressively higher levels of pretest perturbation. As a result, adolescents tended to be less anxious than adults at low to moderate levels of pretest perturbation, but more anxious than adults when the pretest manipulations were more extreme. These across-study comparisons suggest that notably different pretest perturbation may differentially affect adolescents and adults, perhaps resulting in differing conclusions concerning ontogenetic differences in anxiety. Of course, hypotheses derived from across-study comparisons require more systematic investigation within the same study.

The purpose of Experiment 2, therefore, was to directly test the hypothesis that age-related differences in anxiety behavior observed in the EPM would vary according to the level of pretest perturbation experienced by the experimental animals, particularly among adults. In order to assess this possibility, three different pretest conditions were chosen, ranging from minimal perturbation (testing directly upon removal from the home cage), to more extreme pretest manipulations (using pretest conditions similar to those employed in our acute ethanol hangover studies).

2.9. Methods

A total of 120 male and female adolescent and adult Sprague–Dawley rats ($n = 10$ per group) were used across the 2 (age) × 2 (sex) × 3 [pretest: low, moderate or high] factorial design of the experiment. For animals in the low perturbation condition, subjects were removed from the home cage and cagemate, carried the short distance to an adjacent testing room and placed on the center of the EPM for a 5-min session. Rats exposed to moderate levels of pretest perturbation were removed from their home cage and socially isolated in a novel holding cage for 30 min before being carried the short distance to the EPM testing room. Animals in the high perturbation group were subjected to manipulations similar to those used for saline-exposed control animals in our previous studies of ethanol hangover (Doremus-Fitzwater and Spear, 2007; Doremus et al., 2003). Specifically, at approximately 1500–1600 h on the afternoon prior to EPM testing, animals were weighed and administered a saline injection (0.9% w/v) at 2.5% of their body weight (a volume similar to that injected when giving a large 4.0 g/kg ethanol injection). After injection, animals were re-housed with their cagemate in a novel cage (24 × 45.5 × 20 cm) and

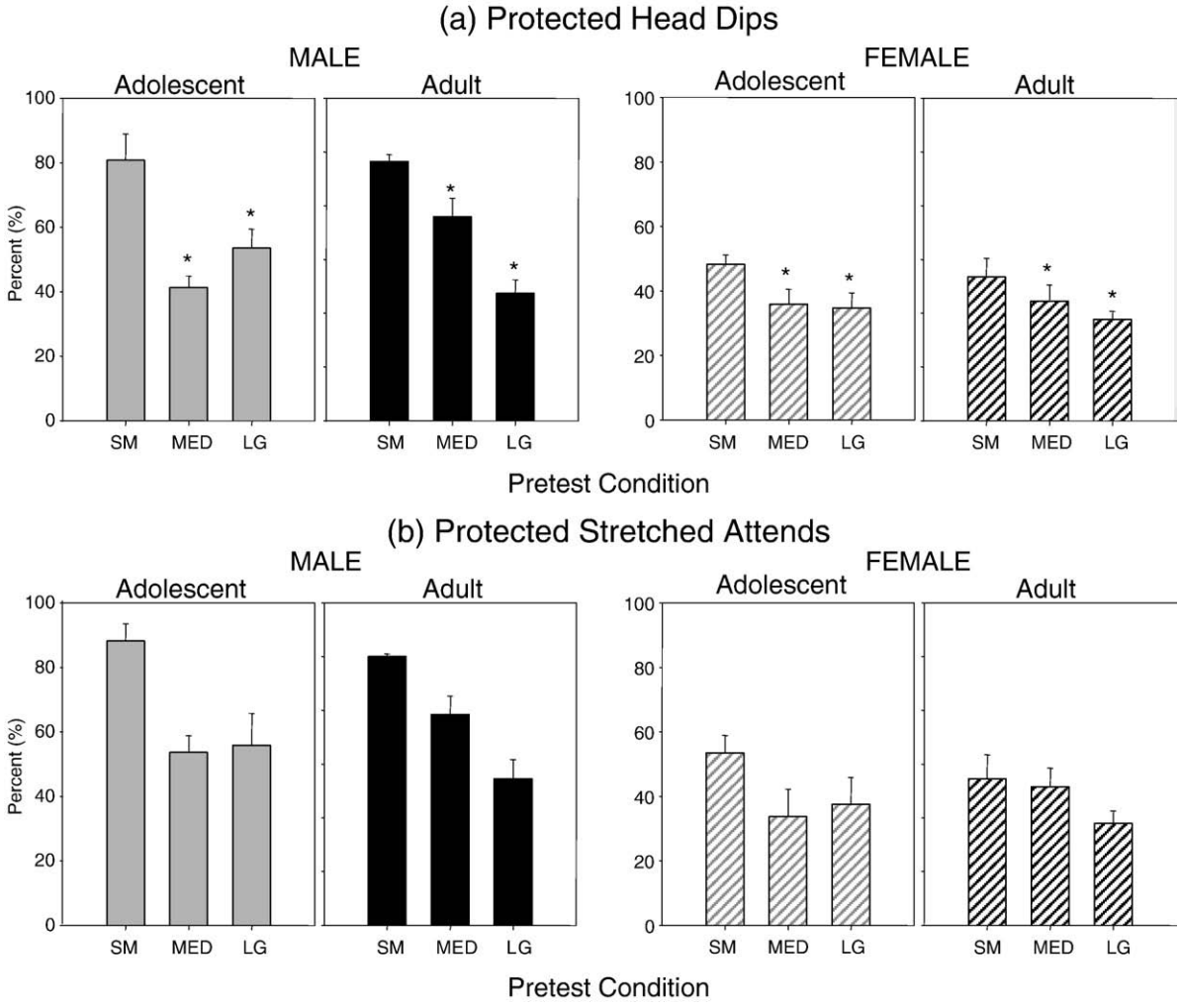

Fig. 5. Percent protected head dips (a) and percent protected stretched attend postures (b) of adolescent and adult male (solid bars) and female (hatched bars) rats in the elevated plus-maze. Animals were tested either directly from the home cage (SM), following a 30-min period of social isolation in a novel environment (MED), or following a 30-min period of social isolation with novelty, preceded by rehousing and a large saline injection 18 h prior to testing (LG).). Asterisks denote a significant difference from the home cage control group and are meant to emphasize the significant age × pretest interaction (i.e. when data were collapsed across sex).

left undisturbed overnight with ad libitum access to food and water. On the following morning (0900–1000 h), animals were then isolated from their partners in a novel holding cage for 30 min prior to EPM testing.

2.10. Results

Two animals fell from the maze during testing and were therefore not included in these analyses: one adult male and female animal, both from the high perturbation condition. Outlier tests revealed that four adolescents (two from the moderate pretest condition and one from each of the other pretest conditions) and four adults (two from the low pretest condition, one from each of the other pretest conditions) met the criterion for exclusion.

2.11. Anxiety

2.11.1. Perturbation-related effects

An influence of pretest conditions on subsequent anxiety levels was evident at both ages for all anxiety-related measures. A significant main effect of pretest was observed for all measures [percent open arm entries: $F(2,98)=21.9$, $p\leq.00001$; percent open arm time: $F(2,98)=21.5$, $p\leq.00001$; percent protected head dips: $F(2,98)=25.7$, $p\leq.00001$; percent protected stretched attend postures: $F(2,98)=15.9$, $p\leq.0001$], reflecting that increased levels of pretest perturbation were accompanied by a subsequent decrease in anxiety. These general anxiolytic effects of greater pretest manipulations, however, were often significantly moderated by age or sex, as outlined below.

2.11.2. Age-related effects

Effects of pretest perturbation on anxiety levels often varied with age. The analyses of percent open arm entries (Fig. 4a), and percent open arm time (Fig. 4b) revealed significant age × pretest interactions [percent open arm entries: $F(2,98)=4.44$, $p=.014$; percent open arm time: $F(2,98)=3.10$, $p=.049$]. Specifically, for both measures, adolescents from the moderate and large pretest perturbation groups were significantly less anxious than adolescents tested directly from the home cage. Adults also showed reductions in anxiety as pretest perturbation increased, but only adults from the large pretest group were significantly less anxious than home cage controls. Age differences in anxiety revealed that only under conditions of moderate pretest perturbation were adolescents significantly less anxious than adults. When anxiety was assessed via percentage of protected head dips (Fig. 5a), the interaction of age and pretest condition was again significant [$F(2,98)=4.50$, $p\leq0.013$]. For this measure, moderate and large amounts of pretest perturbation decreased anxiety for both adolescents and adults when compared to their home cage controls animals. In parallel with the results for open arm activity, a significant age difference in anxiety was observed, with adolescents again being significantly less anxious than adults under conditions of moderate pretest perturbation. While the pattern of results for the age by pretest interaction in the analysis of percent protected stretch attend postures was similar to the other anxiety measures, this effect did not reach significance ($p=.09$) (Fig. 5b).

2.11.3. Sex-related effects

The variable of sex interacted with the effects of pretest perturbation for several of the anxiety measures. In the analysis of percent open arm entries, a sex x pretest interaction emerged [$F(2,98)=4.66$, $p=.012$]. Females were less anxious than males when tested directly from the home cage and also when pretest perturbation was moderate, a sex difference no longer apparent following the large pretest manipulation (Fig. 4a). A sex × pretest interaction [$F(2,98)=5.30$, $p=.007$] also emerged in the analysis of percent protected head dips (Fig. 5a). As was observed with other anxiety measures, females were less anxious than males. Although this sex difference was most pronounced in the low perturbation group, post hoc analyses revealed that this sex difference remained significant under all pretest conditions. In the analysis of percent protected stretched attend postures, a non-significant pattern ($p=.07$) similar to that seen in the analyses of the other anxiety-like behaviors was observed, with sex-related differences in anxiety tending to be more robust under conditions of low pretest perturbation and becoming less pronounced as pretest manipulations were increased (Fig. 5b).

When anxiety levels were assessed via percent open arm time (Fig. 4b) and percent protected stretched attend postures (Fig. 5b), some overall sex differences in anxiety levels were observed, with females generally being less anxious than males [main effect sex for percent open arm time: $F(1,98)=27.43$, $p=.000001$; for percent protected stretched attends: $F(1,98)=43.08$, $p=.00001$].

2.12. Activity data

When the effects of pretest manipulations on activity levels were assessed via closed arm entries (Fig. 6), greater levels of pretest perturbation generally increased activity levels [main effect of pretest:

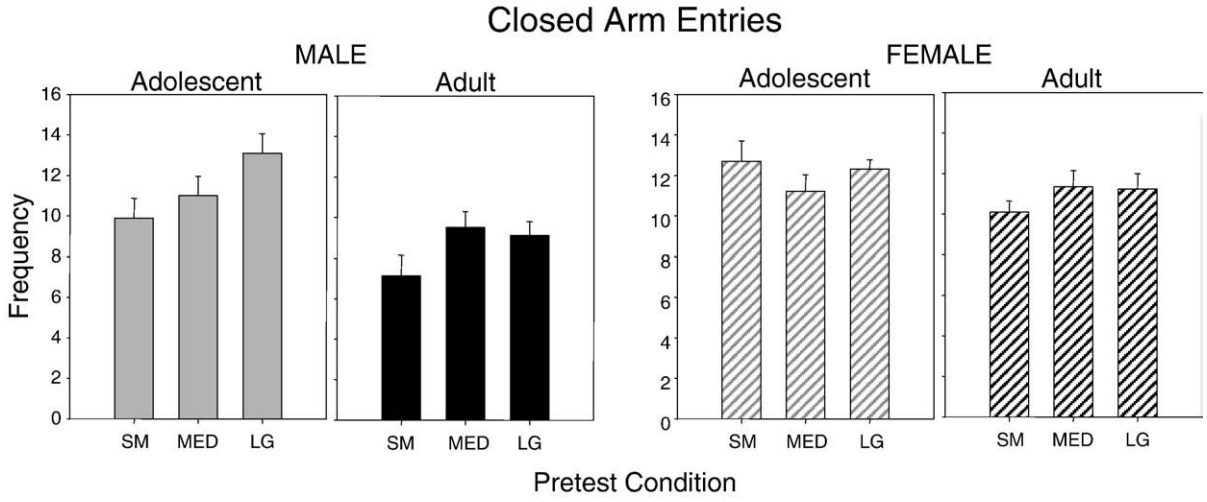

Fig. 6. Total number of closed arm entries exhibited by adolescent and adult male (solid bars) and female (hatched bars) rats in the elevated plus-maze. Animals were tested either directly from the home cage (SM), following a 30-min period of social isolation in a novel environment (MED), or following a 30-min period of social isolation with novelty, preceded by rehousing and a large saline injection 18 h prior to testing (LG).

$F(2,98) = 3.20$, $p \leq .045$]. While this effect appeared most pronounced among male animals, pretest condition did not significantly interact with either age or sex. Overall, however, adolescents were significantly more active than adults [main effect of age: $F(1,98) = 16.73$, $p \leq .0001$], with females being significantly more active than males in this test [main effect sex: $F(1,98) = 10.16$, $p \leq .001$] in the EPM (Fig. 6).

3. Discussion

When the effects of mild pretest manipulations immediately prior to EPM testing were assessed in Exp. 1, both novelty exposure and, to a lesser extent, social isolation resulted in a modest, but significant reduction in anxiety-like behavior regardless of age or sex. When EPM data collected across a variety of experiments were examined, however, it seemed that more extreme levels of pretest perturbation resulted in more dramatic alterations in anxiety levels relative to animals tested directly from the home cage, with these effects differentially expressed between adolescents and adults. Experiment 2 was then conducted to test these across-experiment effects by including moderate and extreme levels of pretest perturbation. These findings revealed anticipated age differences in anxiety, with adults showing a linear decrease in anxiety as pretest perturbation increased, whereas adolescents demonstrated similar levels of anxiety once perturbations were more than minimal. Additional effects of sex were also observed in Exp. 2. In general, females were less anxious than males, however this sex difference in anxiety was more pronounced under minimal pretest perturbation, and became less dramatic as the animals experienced more manipulations prior to EPM testing.

As mentioned earlier, the idea that perturbations prior to EPM testing may influence the expression of anxiety behavior in the maze is certainly not new. Indeed, numerous studies have previously shown that, at least in adults (see Hogg, 1996 for review), manipulations such as prior handling (Schmitt and Hiemke, 1998), transportation to the testing site on a cart (Morato and Brandao, 1996) and housing condition (Haller and Halasz, 1999; Schmitt and Hiemke, 1998) can alter EPM anxiety levels. The results presented in these experiments confirm these earlier studies and demonstrate that even more subtle manipulations (such as a brief period of social isolation or novelty exposure) are sufficient to alter basal anxiety levels in non-drug-exposed rats.

Within the present experiments, the pretest perturbations used resulted in a subsequent decrease in anxiety-like behavior, with more intense levels of perturbation reducing anxiety levels to an even greater extent. While one might expect the opposite (that acute perturbation or stress would results in anxiogenesis), other studies have shown effects similar to those observed here. For example, an episode of acute social defeat resulted in an anxiolytic effect (greater number of open arm entries) in adult male Wistar rats compared to non-stressed controls (Haller et al., 1998). In another study investigating the effects of pretest handling (Schmitt and Hiemke, 1998), 1 week of a mild (5 min per day) handling procedure was found to decrease anxiety-like behaviors in the EPM in two different strains of rats. Furthermore, in studies conducted by another group, the combination of mild social stress with a brief period of isolation (Morato and Brandao, 1997) or the combination of transportation on a cart with brief social isolation (Morato and Brandao, 1996) resulted in an anxiolytic response relative to control animals. Thus, while exposure to seemingly aversive procedures prior to EPM testing might be expected to increase anxiety, there is an increasing consensus that in some situations, pretest perturbations might actually attenuate levels of anxiety. Since greater levels of pretest perturbation were also shown to slightly increase activity levels indexed via number of closed arm entries, and other experimenters have eluded to this effect as well (e.g. Pellow et al., 1985), it is possible that the decreased levels of anxiety observed following moderate or high levels of perturbation are due in part to overall greater levels of maze exploration. To the extent that animals are more active and explore the maze, they may be more likely to sample unprotected areas of the EPM.

Importantly, whereas the pretest manipulations impacted the expression of EPM anxiety in both adolescent and adult animals, they did so differentially across age. Specifically, whereas adults exhibited a linear decrease in anxiety as pretest perturbation increased, this pattern was not evident in adolescents. The results from both the across-experiment comparisons and those from Exp. 2 demonstrated that adolescents show no clear pattern of change in anxiety in relation to alterations in pretest perturbation. In fact, adolescents (unlike adults) seem to show fairly consistent levels of anxiety when tested under pretest conditions ranging from moderate to extreme, suggesting that they might be less susceptible to alterations in baseline anxiety levels due to changes in pretest circumstances. To our knowledge, age differences in the effects of pretest manipulations have not yet been systematically investigated by others. Yet, in one study of 6 week old (i.e. P43) late adolescent rats, EPM behavior of the animals was found to be surprisingly insensitive to the procedural variables examined (including maze construction, swim stress, restraint stress and footshock stress) (Falter et al., 1992). Albeit without adult controls, these data provide additional evidence that adolescents are relatively resistant to changes in EPM anxiety due to exogenous variables.

Not only were age differences apparent in the effects of pretest perturbations, but sex differences emerged as well. Overall, the results of Exp. 2 found females to be less anxious than males. While the direction of sex differences in anxiety seems to vary greatly depending upon the anxiety assay used (see Palanza, 2001), attenuated anxiety in females relative to males when tested in the EPM is a finding that has been reported elsewhere by numerous other researchers (Farabollini et al., 1987; Imhof et al., 1993; Johnston and File, 1991; Lucion et al., 1996; Zimmerberg and Farley, 1993). Although it is possible that sex-related differences in the ratio of body size to maze size may contribute to sex differences in EPM anxiety, it is unlikely that the sex differential is a primary causal factor of the observed sex differences in anxiety. For instance, when Imhof et al. (1993) examined sex differences in anxiety in animals aged P45, P90, P120 and P150, females were found to be less anxious than males at P45 and P90, with this sex difference no longer observed by P120 and P150—ages where the discrepancy between the body size of males and females would be the most pronounced. Furthermore, Johnston and File (1991) investigated sex differences in anxiety in the EPM and observed that females were less anxious than males, even when animals were tested at the same body weight, but at slightly different ages (i.e. P70 in females, P63 in males). Further evidence is also provided by the results of the current study, where the same sex differences in anxiety were generally observed among adolescents at an age when the sexes have not yet significantly diverged in body weight (see Vetter and Spear, 2007).

It is also possible that expression of anxiety among females may vary according to phase of the estrous cycle, potentially contributing to our observation of sex differences in the EPM. Indeed, several researchers have reported that anxiety levels in the EPM change across the reproductive cycle, with females generally less anxious during proestrus compared to diestrus (Diaz-Veliz et al., 1997; Frye et al., 2000; Marcondes et al., 2001; Mora et al., 1996). In contrast, however, other groups have failed to find a significant effect of estrous cycle on anxiety-like behavior (Bitran et al., 1991; Byrnes and Bridges, 2006; McCormick et al., 2008; Stock et al., 2000). In the context of the current study, if stage of the estrous cycle impacted expression of anxiety among adult females but not their adolescent counterparts, it would be expected that data from adult females would have been more variable than among the other groups. Consequently, it would also be expected that age might have interacted significantly with sex in the analyses of anxiety-like behaviors. There was no evidence for either of these possibilities, however, suggesting that data from these

studies were not notably influenced by estrous cyclicity among the adult females.

When sex differences in anxiety were considered under a variety of pretest conditions in the current study, a pattern emerged in which sex differences were most apparent under low levels of pretest perturbation (i.e. when baseline levels of anxiety were the highest), with these differences becoming less pronounced as levels of pretest manipulations increased. Past research examining the effects of acute and/or chronic stress on anxiety-like behavior in the EPM using both male and female rodents has certainly found sex differences in these effects (e.g. see Albonetti and Farabollini, 1992; Chadda and Devaud, 2005; McCormick et al., 2008; Steenbergen et al., 1991), although there has been surprisingly little emphasis on possible sex differences in the effects of more methodological and procedural variables on the expression of baseline anxiety measures in the EPM. Our current results would suggest that even when conducting research in adult animals, the influence of pretest conditions on anxiety need to be considered when including both male and female animals, especially when the ultimate goal is to examine possible sex differences in the effects of pharmacological manipulations on anxiety.

Taken together, the results of these experiments have highlighted the lability of anxiety-like behaviors in the EPM to pretest procedural variables, with these effects being dependent on both age and sex of the animals tested. These results, caution against drawing strong conclusions from EPM data regarding age differences in basal anxiety levels without confirmation using other anxiety tests that appear less susceptible to pretest perturbations, such as the social interaction test. When using the EPM to investigate effects of pharmacological manipulations, however, it may be possible to exploit particular pretest conditions to perhaps increase sensitivity of the EPM for the manipulation of interest. For example, when assessing potential anxiolytic effects of drugs or conditions, the high baseline anxiety associated with testing animals directly from the home cage may be desirable. Conversely, anxiogenic effects of pharmacological compounds or experimental conditions may be more readily observed under low levels of basal anxiety that are somewhat paradoxically promoted by testing animals following more extreme pretest perturbations. Certainly, when comparing findings regarding age or sex differences in anxiolytic and anxiogenic effects, careful consideration of pretest/test procedures and resultant baseline anxiety levels may provide important clues concerning likely contextual and procedural contributors to differential findings across studies.

Acknowledgements

Supported by grants R01 DA019071, R37 AA12525 and R01 AA01735501 to Linda Spear and R01 AA12453 to Elena Varlinskaya.

References

Adriani W, Granstrem O, Macri S, Izykenova G, Dambinova S, Laviola G. Behavioral and neurochemical vulnerability during adolescence in mice: studies with nicotine. Neuropsychopharmacology 2004;29:869–78.

Albonetti ME, Farabollini F. Behavioral responses to single and repeated restraint in male and female rats. Behav Processes 1992;28:97-110.

Andrade MM, Tome MF, Santiago ES, Lucia-Santos A, de Andrade TG. Longitudinal study of daily variation of rats' behavior in the elevated plus-maze. Physiol Behav 2003;78:125–33.

Bitran D, Hilvers RJ, Kellogg CK. Ovarian endocrine status modulates the anxiolytic potency of diazepam and the efficacy of gamma-aminobutyric acid-benzodiazepine receptor-mediated chloride ion transport. Behav Neurosci 1991;105:653–62.

Byrnes EM, Bridges RS. Reproductive experience alters anxiety-like behavior in the female rat. Horm Behav 2006;50:70–6.

Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 2005;29:1193–205.

Chadda R, Devaud LL. Differential effects of mild repeated restraint stress on behaviors and GABA(A) receptors in male and female rats. Pharmacol Biochem Behav 2005;81:854–63.

Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav 1994;49:171–6.

Degroot A, Wade M, Salhoff C, Davis RJ, Tzavara ET, Nomikos GG. Exposure to an elevated platform increases plasma corticosterone and hippocampal acetylcholine in the rat: reversal by chlordiazepoxide. Eur J Pharmacol 2004;493:103–9.

Diaz-Veliz G, Alarcon T, Espinoza C, Dussaubat N, Mora S. Ketanserin and anxiety levels: influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats. Pharmacol Biochem Behav 1997;58:637–42.

Doremus-Fitzwater TL, Spear LP. Developmental differences in acute ethanol withdrawal in adolescent and adult rats. Alcohol Clin Exp Res 2007;31:1516–27.

Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Anxiolytic effects of diazepam in adolescent and adult rats: impact of social versus non-social testing. Paper presented at International Society for Developmental Psychobiology, Atlanta, GA; 2006. October.

Doremus TL, Brunell SC, Varlinskaya EI, Spear LP. Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. Pharmacol Biochem Behav 2003;75:411–8.

Doremus TL, Varlinskaya EI, Spear LP. Factor analysis of elevated plus-maze behavior in adolescent and adult rats. Pharmacol Biochem Behav 2006;83:570–7.

Espejo EF. Structure of mouse behavior on the elevated plus-maze test of anxiety. Behav Brain Res 1997;86:105–12.

Falter U, Gower AJ, Gobert J. Resistance of baseline activity in the elevated plus-maze to exogenous influences. Behav Pharmacol 1992;3:123–8.

Farabollini F, File SE, Johnston AL, Wilson CA. An analysis of sex differences in the open field and tests of exploration and anxiety. Br J Pharmacol 1987;90:263P.

Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol Biochem Behav 1996;54:31–40.

Foa EB, Costello EJ, Franklin M, Kagan J, Kendall P, Klein R, et al. Anxiety disorders (Report of the Annenberg Adolescent Anxiety Disorders Commission). In: Evans DL, Foa EB, Gur RE, Hendin H, O'Brien CP, Seligman MEP, Walsh BT, editors. Treating and preventing adolescent mental health disorders. New York: Oxford University Press, Inc.; 2005. p. 159–253.

Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha,5alpha-THP. Pharmacol Biochem Behav 2000;67:587–96.

Haller J, Halasz J. Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity. Psychopharmacology (Berl) 1999;144:311–5.

Haller J, Halasz J, Makara GB, Kruk MR. Acute effects of glucocorticoids: behavioral and pharmacological perspectives. Neurosci Biobehav Rev 1998;23:337–44.

Hascoet M, Colombel MC, Bourin M. Influence of age on behavioural response in the light/dark paradigm. Physiol Behav 1999;66:567–70.

Hefner K, Holmes A. Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. Behav Brain Res 2007;176:210–5.

Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 1996;54:21–30.

Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicol Teratol 1992;14:221–8.

Imhof JT, Coelho ZM, Schmitt ML, Morato GS, Carobrez AP. Influence of gender and age on performance of rats in the elevated plus maze apparatus. Behav Brain Res 1993;56:177–80.

Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. Guide for care and use of laboratory animals. Washington, DC: National Academy Press; 1996.

Johnston AL, File SE. Sex differences in animal tests of anxiety. Physiol Behav 1991;49:245–50.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 2005;62:593–602.

Lal H, Prather PL, Rezazadeh SM. Anxiogenic behavior in rats during acute and protracted ethanol withdrawal: reversal by buspirone. Alcohol 1991;8:467–71.

Lucion AB, Charchat H, Pereira GA, Rasia-Filho AA. Influence of early postnatal gonadal hormones on anxiety in adult male rats. Physiol Behav 1996;60:1419–23.

Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC. Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiol Behav 2001;74:435–40.

McCormick CM, Smith C, Mathews IZ. Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. Behav Brain Res 2008;187:228–38.

Mora S, Dussaubat N, Diaz-Veliz G. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 1996;21:609–20.

Morato S, Brandao ML. Transporting rats to the test situation on a cart can modify rat exploratory behavior in the elevated plus-maze. Psychobiology 1996;24:247–52.

Morato S, Brandao ML. Paradoxical increase of exploratory behavior in the elevated plus-maze by rats exposed to two kinds of aversive stimuli. Braz J Med Biol Res 1997;30:1113–20.

Palanza P. Animal models of anxiety and depression: how are females different. Neurosci Biobehav Rev 2001;25:219–33.

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.

Prather PL, Rezazadeh SM, Lane JD, Rowan GA, Hooper ML, Lytle DA, et al. Conflicting evidence regarding the efficacy of ondansetron in benzodiazepine withdrawal. J Pharmacol Exp Ther 1993;264:622–30.

Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology, and ethology. In: Cooper SJ, Hendrie CA, editors. Ethology and psychopharmacology. Chichester: John Wiley & Sons, Ltd.; 1994. p. 9-44.

Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev 1997;21:801–10.

Schmitt U, Hiemke C. Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling. Pharmacol Biochem Behav 1998;59: 807–11.
Slawecki CJ. Comparison of anxiety-like behavior in adolescent and adult Sprague–Dawley rats. Behav Neurosci 2005;119:1477–83.
Slawecki CJ, Roth J. Comparison of the onset of hypoactivity and anxiety-like behavior during alcohol withdrawal in adolescent and adult rats. Alcohol Clin Exp Res 2004;28:598–607.
Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.
Steenbergen HL, Farabollini F, Heinsbroek RP, Van de Poll NE. Sex-dependent effects of aversive stimulation on holeboard and elevated plus-maze behavior. Behav Brain Res 1991;43:159–65.
Stock H, Foradori C, Ford K, Wilson MA. A lack of tolerance to the anxiolytic effects of diazepam on the plus-maze: comparison of male and female rats. Psychopharmacology (Berl) 2000;147:362–70.
Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. Alcohol Clin Exp Res 2002;26: 1502–11.
Varlinskaya EI, Spear LP. Social interactions in adolescent and adult Sprague–Dawley rats: impact of social deprivation and test context familiarity. Behav Brain Res 2008;188:398–405.
Vetter CS, Spear LP. Age-associated trajectories of consumption and body weight gain in pair- and isolate-housed adolescent and adult Sprague–Dawley rats. Paper presented at International Society for Developmental Psychobiology, San Diego, CA; 2007. November.
Wada T, Fukuda N. Effects of DN-2327, a new anxiolytic, diazepam and buspirone on exploratory activity of the rat in an elevated plus-maze. Psychopharmacology (Berl) 1991;104:444–50.
Walker EF, Walder DJ, Reynolds F. Developmental changes in cortisol secretion in normal and at-risk youth. Dev Psychopathol 2001;13:721–32.
Walker FR, March J, Hodgson DM. Endotoxin exposure in early life alters the development of anxiety-like behaviour in the Fischer 344 rat. Behav Brain Res 2004;154:63–9.
Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev 2001;25:275–86.
Wallis CJ, Lal H. A discriminative stimulus produced by 1-(3-chlorophenyl)-piperazine (mCPP) as a putative animal model of anxiety. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:547–65.
Wilmouth CE, Spear LP. Withdrawal from chronic nicotine in adolescent and adult rats. Pharmacol Biochem Behav 2006;85:648–57.
Wilson MA, Burghardt PR, Ford KA, Wilkinson MB, Primeaux SD. Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. Pharmacol Biochem Behav 2004;78:445–58.
Zimmerberg B, Farley MJ. Sex differences in anxiety behavior in rats: role of gonadal hormones. Physiol Behav 1993;54:1119–24.