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Factor analysis of elevated plus-maze behavior in adolescent and adult rats

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Abstract

The elevated plus-maze (EPM) is among the most popular behavioral models of anxiety. While numerous experiments have validated this test hormonally, pharmacologically, and with factor analysis in adult rodents, few studies have explored use of the EPM to examine ontogenetic differences in anxiety. Given the growing interest in adolescence and the mixed findings to date regarding age-related differences in anxiety, validation of the EPM model for use in adolescence is important. Therefore, the present experiment employed factor analysis to examine underlying EPM behavioral components in adolescent and adult male and female Sprague–Dawley rats across three separate data sets. Results of the analyses conducted across both age and sex produced a 3-factor solution, with the primary component of EPM behavior consisting of anxiety-related behaviors in both adolescent and adult males and females. Within the age analyses, the second and third factors were comprised largely of activity- and risk-related behaviors, respectively. Sex analyses revealed a similar pattern in females, with some behaviors comprising the second and third factors reversed in males. Taken together these results confirm use of the EPM in adolescent and adult rats and demonstrate slight differences in the underlying components of EPM behavior in males versus females.

Keywords: Rat; Adolescent; Elevated plus-maze; Factor analysis; Anxiety

1. Introduction

In recent years, there has been a growing research interest in adolescence as a highly conserved developmental period. Although previously attributed uniquely to humans, adolescence has now been characterized as a transition seen across many mammalian species (Adriani and Laviola, 2004; Spear, 2000). In addition to the hormonal, neural and psychosocial transformations observed during this ontogenetic period, certain age-typical behavioral characteristics, such as increases in risk-taking, sensation-seeking, and novelty seeking behaviors, are commonly observed in adolescents of a variety of species (Spear, 2000).

Some researchers have hypothesized that adolescent-typical increases in risk-taking and related behaviors may be associated with developmental alterations in anxiety. Research is sparse using animal models of adolescence to examine the ontogeny of anxiety, and available findings are mixed as to whether adolescent animals are more or less anxious than their adult counterparts. For example, adolescent male mice (Hascoet et al., 1999) and rats (Slawecki, 2005) were reported to exhibit heightened anxiety relative to adults when tested in a light/dark box. Similarly, in prior work in our laboratory, adolescent saline control animals were found to be more anxious than their adult counterparts when tested using an elevated plus maze (EPM) test for anxiety (Doremus et al., 2003a). However, in other experiments using the social interaction test (Varlinskaya and Spear, 2002) and the open field test (Slawecki and Roth, 2004), no age-related differences in baseline anxiety levels were observed. Yet other studies have reported that anxiety levels are lower during adolescence, with anxiety increasing across age in rats in one report (Imhof et al., 1993) and anxiety levels reported to be lower in late-adolescent mice when compared to younger and older animals in work by another group (Macri et al., 2002). Most likely, methodological differences across laboratories — such as

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age at testing, type of test used to index anxiety, pretest manipulations, and specific test procedures — are responsible for these conflicting ontogenetic findings.

In spite of the many animal models available to assess anxiety levels, the EPM is by far one of the most widely used. The EPM consists of a plus-shaped apparatus elevated above the floor with two open and two enclosed arms joined by a central square. The fear of open elevated spaces conflicts with a desire to explore novel areas, with more time and entries onto the open arms used to index relatively low anxiety levels. Behaviorally and hormonally, the EPM has been validated as a model of anxiety/fear, since confinement to the open arms elevated corticosterone levels and resulted in increased fear-related behaviors when compared to animals restricted to the closed arms (Pellow et al., 1985). Previous research has also pharmacologically validated this model in adults: classic anxiolytic compounds (e.g. diazepam) increased the percentage of entries and time spent on the open arms and anxiogenic compounds (e.g. pentylenetetrazol) decreased these percentages, whereas drugs not associated with anxiety (e.g. haloperidol) failed to affect percent open arm time/entries (Pellow et al., 1985; Pellow and File, 1986; for review see Rodgers and Cole, 1994). Yet another advantage of the EPM is that it has been validated across species, with similar pharmacological responses and behavioral profiles observed in both mice and rats (Lister, 1987).

In further support of this test as a behavioral model of anxiety, factor analysis has been employed to validate the underlying components of behavior in the EPM (for reviews see Rodgers and Dalvi, 1997; Wall and Messier, 2000). Although 2-factor up to 5-factor models have been reported across these analyses, generally the first factor (i.e. the factor that accounts for the largest amount of variance) is comprised of anxiety measures (at least in male rodents), with behaviors such as percentage of open arm entries and time (Cruz et al., 1994; Fernandes and File, 1996), as well as the more ethologically relevant behaviors of percentage of protected head dips and protected stretched attends (Rodgers and Dalvi, 1997; Rodgers and Johnson, 1995), loading highly on this factor. Behaviors loading on the second factor often consist of activity measures, such as number of closed arm entries and total arm entries (Cruz et al., 1994; Fernandes et al., 1999; Rodgers and Dalvi, 1997). Factor analysis techniques have additionally shown that the characteristics of EPM behavior are qualitatively changed when animals are given repeated exposures to this test (File et al., 1993; File and Zangrossi, 1993; Holmes and Rodgers, 1998; Ouagazzal et al., 1999). Thus, while responses to classic anxiolytic drugs are changed in subsequent trials of the EPM test, so also are the underlying behavioral components (Fernandes and File, 1996; File et al., 1993).

Despite the wealth of research that has been conducted using the EPM behavioral test of anxiety, to our knowledge factor analysis has not yet been employed to validate the use of this test as an index of anxiety in animals other than adults. To the extent that the plus-maze is used to examine anxiety levels in adolescent as well as adult rats, then it is important to determine whether this test reflects the same underlying components of behavior in rats of both ages. Therefore, the purpose of the present experiments was to subject behavioral data obtained from both adolescent and adult rats in three separate studies to principal components analysis to compare and contrast factors underlying EPM behavior in adolescent rats with comparably treated adult animals.

In addition to these age analyses, another purpose of this study was to examine EPM behavior of both male and female adolescent and adult rats, since few studies have employed factor analysis to explore behavioral components in animals of both sexes. A previous factor analysis study of male and female adult rats yielded a 2-factor solution, with females reported to have opposite underlying EPM behavioral components compared to males (Fernandes et al., 1999). Specifically, anxiety measures for males loaded on the primary behavioral component, with measures thought to index activity loading on the second factor. In contrast, female behavior was primarily driven by activity-like behaviors and secondarily by typical anxiety behaviors. Therefore, inclusion of the present sex analyses helped to further characterize and expand our understanding of apparent sex differences previously reported in this behavioral assay of anxiety.

2. General methods

2.1. Subjects

A total of 186 Sprague–Dawley rats derived from three previous experiments conducted in our lab was used for these analyses, as described below. Animals were bred in our colony and on the day after birth, postnatal day 1 (P1), litters were culled to 8–10 pups, with 6 animals of one sex and 4 animals of the other being retained whenever possible. Male and female offspring were weaned at P21 and housed in same-sex littermate pairs in 18×24×18 cm cages. At P55, adult male pairs were moved to larger cages (24×40.5×18 cm) until the time of testing. All animals were maintained in a temperature-controlled vivarium on a 14:10-h light:dark cycle (lights on 0700 h), with ad libitum access to water and food (Purina Rat Chow, Lowell, MA). No more than one animal per litter was placed in any given experimental group as defined by the factorial design of each experiment. At all times, rats used in these experiments were maintained and treated in accordance with 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).

2.2. Apparatus

The adult elevated plus maze (EPM) consisted of two 48.26×12.7 cm open arms and two 48.26×12.7×29.21 cm closed arms. The adolescent EPM was proportionately sized based on crown–rump length and confirmed by gait analysis, and consisted of 30×8.89 cm open arms and 30×8.89×20.32 cm closed arms. Because our laboratory often examines animals following ethanol exposure, and intoxicated animals lose some of their 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. While the addition of ledges to the open arms of the maze has been found to impact plus-maze behavior, the effect of ledges on the primary underlying EPM behavioral components during the animals' first exposure to the test seems modest (Fernandes and File, 1996). Gaps of 4.0 cm (adolescent) and 4.5 cm (adult) at the junctions of the open and closed arms provided easy access below the plane of the maze to allow for protected head dips over the sides of the maze. Both mazes were elevated to a height of 50 cm. All sessions were conducted under dim light (3 lx), with a white noise generator used to attenuate superfluous sounds during testing. Sessions were videotaped by a camera mounted at a height of 147 cm above the apparatus to allow testing of the animals without an experimenter present in the room. After testing each animal, the apparatus was cleaned with a 3% hydrogen peroxide solution and dried before the next animal was placed on the apparatus.

2.3. General testing procedures

Although pretest procedures differed across experiments, test procedures in the EPM were held constant. 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: open (OAT) and closed arm time (CAT), open (OAE) and closed arm entries (CAE), number of protected (PHD) and unprotected head dips (UHD), number of protected (PSAP) and unprotected stretched attend postures (USAP), and number of rears. An animal was considered to have entered an arm when all four paws were placed in the arm. An animal was considered to have exited an arm when at least two front paws were placed outside the arm. Protected head dips included dipping the head over the sides of the maze from within the center platform or a closed arm, whereas unprotected head dips were considered when the animal dipped its head over the sides of the maze while on an open arm. Protected stretched attends were defined as when the animal's two hind feet remained in a closed arm or the center platform while the animal elongated its head and shoulders, followed by subsequent retraction. An unprotected stretched attend was defined as the same behavior, but when the animal was located on one of the open arms.

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). More recently, percent protected head dips and percent protected stretched attend postures have been suggested to be even more sensitive measures of anxiety, based on ethological analysis and pharmacological manipulations (Espejo, 1997; Rodgers and Cole, 1994; for review and rationale see: Carobrez and Bertoglio, 2005; Cruz et al., 1994; Rodgers and Dalvi, 1997). Closed arm entries and number of rears have been considered indices of activity (Cruz et al., 1994; Rodgers and Dalvi, 1997).

2.4. Data analysis

EPM data from each experiment was separately subjected to factor analysis (for methods see: Fernandes and File, 1996; Rodgers and Johnson, 1995). All test variables included in these analyses were checked for violations of normality. Skewness and kurtosis statistics were evaluated, with data resulting in a statistic of 2 times or less the standard error considered acceptable for analysis. When a particular variable violated this criterion, that measure was subject to transformation (e.g. square root, log $(n+1)$, arc sine), using the transform producing the most normal distribution for that variable. A principal components analysis was then conducted, with an orthogonal (varimax) rotation used on the factor matrix. Only components with an Eigenvalue of 1 or greater were retained for final rotation. Additionally, the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were analyzed to ensure that these data were adequate for use in the analyses. In order to maintain appropriate levels of sampling adequacy (and because of the modest size of these data sets given that only saline-exposed or animals without drug manipulations were included), age analyses were conducted on data collapsed across sex, whereas sex analyses were conducted on data collapsed across age.

2.5. Specific methods

2.5.1. Data set 1

Data set 1 was derived from a study used to examine the influence of different EPM pretest circumstances on consequent plus-maze behavior in both adolescent (P33–35) and adult (P70–75) male and female rats (Doremus et al., 2003b). The design of this experiment was a 2 (age) × 2 (sex) × 3 (pretest: home cage vs. social isolation vs. novel environment) factorial, with all 93 animals from this study included in the factor analyses. Animals in the home cage pretest condition were removed from their home cage, carried a short distance to the adjacent EPM room, and placed directly on the apparatus for the 5 min test session, where testing occurred between 1000 and 1200 h. The cage mate of each of these animals remained in isolation for 30 min in its home cage prior to standard EPM testing (the social isolation pretest condition). For the novel environment pretest condition, a pair of animals were removed from their home cage and placed in a novel breeder tub (24 × 45.5 × 20 cm) with clean pine shavings for 30 min, with one animal from the pair randomly selected for testing on the EPM immediately thereafter.

2.5.2. Data sets 2a and 2b

These data sets included data from control male (data set 2a) and female (data set 2b) animals derived from two studies that examined age-related differences between adolescents (P33–35) and adults (P70–75) in ethanol withdrawal-induced elevations in anxiety ($N=45$). In both studies, these control animals were injected intraperitoneally (i.p.) with 0.9% saline (*w*/*v*) at a volume of 2.52% of body weight and tested on the EPM 12–18 h post-injection (between 0800 and 1200 h), with the timing based

on testing of the experimental animals 3 h following ethanol clearance (18 h for adult males, 13 h for adult females, and 12 h for both male and female adolescents). The study used to provide data set 2a (Doremus et al., 2003a) had 2 pretest conditions, with half of the animals placed into size-adjusted (8.57 cm D.× 21.59 cm for adults and 5.08 cm D.×12.70 cm for adolescents) restraint tubes (Braintree Scientific, Inc., Braintree, MA) for 30 min, while the remaining animals were isolated in novel plastic tubs for the 30 min pretest interval. All animals used for the second data set (2b) were subject only to the latter pretest procedure (Doremus and Spear, 2004).

2.5.3. Data set 3

The third data set consisted of saline control animals (N=48) derived from an experiment designed to assess the impact of several acute doses of ethanol in combination with social isolation on subsequent behavior of both adolescent (P35) and adult (P70) male and female rats on the EPM (Varlinskaya and Spear, 2001). Animals were injected i.p. with 0.9% saline at 1.0% of their body weight 30 min prior to the EPM test (1700–1900 h). Half of the animals were placed back into their home cage with their housing partner for the 30 min pre-test interval, while the remaining animals were socially isolated in novel breeder tubs for 30 min prior to EPM testing.

3. Results

In all three data sets and in both the age and sex analyses, the behavioral measure, %OAT, violated the assumption of normality and was therefore subjected to a square root transformation, and is so noted in all tables. Sampling adequacy for data set 1 was particularly robust (see Tables 1 and 4), presumably because all experimental animals were used in that analysis. Sampling adequacies for data sets 2a, 2b and 3 were adequate, albeit marginally so (see Tables 2, 3, 5, 6), likely due to the inclusion of only control (i.e. saline-treated) animals.

Table 1
Factor loadings of elevated plus-maze behaviors of both adolescent and adult rats from data set 1

Data set 1						
Behavior	Factor 1		Factor 2		Factor 3	
	ADOL	Adult	ADOL	Adult	ADOL	Adult
%OAE	**.913**	**.929**	−.224	.209	−.050	−.118
√%OAT	**.983**	**.943**	−.040	.277	−.033	−.020
%PHD	**−.941**	**−.895**	.040	.139	.093	−.096
%PSAP	**−.932**	**−.882**	−.009	−.122	−.091	−.079
CAE	.025	.123	**.894**	**.700**	.306	**.636**
TAE	**.646**	**.548**	**.662**	**.681**	.243	.414
Rears	−.249	.058	**.784**	.082	−.222	**.677**
THD	**.902**	**.633**	.142	**.665**	.036	−.123
TSAP	.444	.110	.048	.252	**−.654**	**−.637**
%HUB	.204	−.021	.142	**.868**	**.785**	−.221
% variance	50.9	40.7	19.5	23.7	12.7	15.3
Percentage of total variance	ADOL=83.0% Adult=79.7%					
Sampling adequacy	ADOL .72 Adult .72					

Factor loadings for both adolescent (ADOL) and adult rats (collapsed across sex) from data set 1 are listed, with loadings higher than 0.5 (or less than −0.5) enlarged and boldfaced for emphasis. Behaviors analyzed in this data set (and all subsequent data sets) were: percentage of open arm entries (%OAE), square root of percent open arm time (√%OAT), percentage of protected head dips (%PHD) and stretched attend postures (%PSAP), number of closed arm entries (CAE), number of total arm entries (TAE), number of rears (Rears), total number of head dips (THD), total number of stretched attend postures (TSAP), and percentage of time spent in the central hub (%HUB).

Table 2
Factor loadings of elevated plus-maze behaviors of both adolescent and adult rats from data set 2

Data set 2						
Behavior	Factor 1		Factor 2		Factor 3	
	ADOL	Adult	ADOL	Adult	ADOL	Adult
%OAE	**.948**	**.933**	.128	−.192	−.112	.169
√%OAT	**.864**	**.940**	.377	.133	−.207	.231
%PHD	−.362	**−.911**	.059	−.021	**.735**	.159
%PSAP	**−.940**	**−.862**	.067	−.027	−.094	−.187
CAE	.097	.229	**.930**	**.890**	.155	.064
TAE	**.578**	**.628**	**.777**	**.689**	−.022	.106
Rears	.280	−.055	−.065	**.746**	**.689**	.293
THD	**.797**	**.620**	.289	.279	.222	**.600**
TSAP	.098	−.372	**.768**	**.769**	−.364	−.215
%HUB	.069	.081	**.704**	.052	**.562**	**.953**
% variance	37.3	43.1	28.1	25.5	16.0	15.6
Percentage of total variance	ADOL=81.4% Adult=84.1%					
Sampling adequacy	ADOL .63 Adult .58					

Factor loadings for both adolescent (ADOL) and adult rats (collapsed across sex) from data set 2 are listed, with loadings higher than 0.5 (or less than −0.5) enlarged and boldfaced for emphasis. Abbreviations for behaviors analyzed are described in Table 1.

3.1. Age-related differences in EPM behavior

As shown in Tables 1–3, three factors emerged consistently from the age analyses across all data sets. The percentage of the total variance accounted for by each of the three factors was similar across age groups and data sets, with this total variance ranging from 78% to 84%.

The more traditional measures of anxiety, %OAE and % OAT, loaded highly and positively on factor 1 in both adolescent and adult animals within each of the three data sets (Tables 1–3). Likewise, the measures of %PHD and % PSAP generally loaded highly and negatively on factor 1 for both adolescents and adults in each data set, with the exception of %PHD for adolescents in data set 2. In addition to these anxiety measures, several other behaviors also loaded on factor 1: total number of head dips (THD) loaded highly in all three data sets, as did total arm entries (TAE) in all groups and data sets except for adolescent animals from the third data set. Across all three data sets and both age groups, CAE and TAE consistently and strongly loaded on factor 2. Percentage of time spent in the central hub (%HUB) also loaded strongly on this factor four times among the six possible combinations of age×data set, whereas number of rears and total stretched attend postures (TSAP) loaded three and two times, respectively. The variability in the loading of these additional

Table 3
Factor loadings of elevated plus-maze behaviors of both adolescent and adult rats from data set 3

Data set 3						
Behavior	Factor 1		Factor 2		Factor 3	
	ADOL	Adult	ADOL	Adult	ADOL	Adult
%OAE	**.859**	**.950**	.030	.067	.094	.081
√%OAT	**.895**	**.912**	.347	.273	.082	.151
%PHD	**−.690**	**−.742**	−.318	.105	−.059	−.122
%PSAP	**−.845**	**−.765**	−.064	−.124	.159	.318
CAE	.066	.090	**.964**	**.954**	.085	−.043
TAE	.446	**.544**	**.846**	**.806**	.075	−.021
Rears	−.089	−.380	.014	**.780**	**.890**	.297
THD	**.809**	**.823**	.418	.327	.117	.211
TSAP	.164	.155	.269	.117	**.787**	**.929**
%HUB	.227	.387	**.703**	**.560**	.188	.124
% variance	36.7	41.7	26.1	27.1	15.2	11.5
Percentage of total variance	ADOL=78.0% Adult=80.3%					
Sampling adequacy	ADOL .66 Adult .57					

Factor loadings for both adolescent (ADOL) and adult rats (collapsed across sex) from data set 3 are listed, with loadings higher than 0.5 (or less than −0.5) enlarged and boldfaced for emphasis. Abbreviations for behaviors analyzed are described in Table 1.

Table 4
Factor loadings of elevated plus-maze behaviors of both male and female rats from data set 1

Data set 1						
Behavior	Factor 1		Factor 2		Factor 3	
	Male	Female	Male	Female	Male	Female
%OAE	**.934**	**.950**	.141	.042	−.015	.094
√%OAT	**.948**	**.963**	.232	.177	.110	.089
%PHD	**−.888**	**−.943**	.188	−.046	−169	−.032
%PSAP	**−.857**	**−.937**	−.221	−.156	−.230	−.014
CAE	.217	.039	**.557**	**.936**	**.726**	−.128
TAE	**.670**	.472	.490	**.838**	.496	−.054
Rears	−.013	−.386	−.166	**.634**	**.859**	.330
THD	**.816**	**.774**	.477	.495	.045	.090
TSAP	**.529**	.169	.021	−.058	−.286	**.946**
%HUB	.085	.263	**.924**	**.634**	−.071	−.013
% variance	47.4	46.7	18.2	26.9	16.9	10.5
Percentage of total variance	Male=82.6% Female=84.1%					
Sampling adequacy	Male .73 Female .75					

Factor loadings for both male and female rats (collapsed across age) from data set 1 are listed, with loadings higher than 0.5 (or less than −0.5) enlarged and boldfaced for emphasis. Abbreviations for behaviors analyzed are described in Table 1.

measures across data sets may be related in part to the marginal sampling adequacy seen with some of the data sets.

A third factor also emerged in all three studies. Behaviors that loaded on factor 3 three or more times in these analyses were: rears, TSAP, and %HUB, with THD, CAE and %PHD emerging once each. Results for this factor were not as consistent across age and data sets as were factors 1 and 2, and typically accounted for only 11–16% of the total variance.

3.2. Sex-related differences in EPM behavior

In order to examine sex differences in the underlying components of EPM behavior, the same data sets analyzed above were collapsed across age prior to analyses conducted for each sex. As shown in Tables 4–6, in all three of these data sets a 3-factor solution emerged, with the percentage of the variance accounted for by each factor approximately equivalent across sex and factors 1–3 accounting for 76% to 87% of the total variance across these experiments.

Contributors to the first factor consisted primarily of anxiety-like behaviors, such as %OAE, %OAT, %PSAP and %PHD, with these measures generally loading strongly for both males and females in all three data sets (although %PHD did not strongly load with this factor among females in data set 2). Similar to the age analyses, THD also loaded highly on this factor for both sexes in all data sets. Additionally, TAE loaded twice across the six sex data set combinations, whereas TSAP and %HUB each loaded once.

Data sets 1–3 showed that CAE loaded highly on factor 2 for both male and female animals. Among females, additional activity-related measures also loaded on this factor, including TAE in all three data sets, and with number of rears and %HUB loading highly among females in two of three instances and TSAP showing a robust factor 2 loading in one case. Contributors to factor 2 emerged less consistently in male animals. CAE was the only behavioral measure to load strongly across the three data sets, whereas %HUB showed robust factor loadings in two of the three data sets among males, and THD, TSAP and TAE each loaded highly in only one instance.

In male rats, number of rears loaded heavily on factor 3 in all three data sets, CAE loaded highly in two data sets, and TAE and TSAP each loaded strongly in only one data set, suggesting

Table 5
Factor loadings of elevated plus-maze behaviors of both male and female rats from data set 2

Data set 2						
Behavior	Factor 1		Factor 2		Factor 3	
	Male	Female	Male	Female	Male	Female
%OAE	**.949**	**.948**	−.046	.042	−.151	−.166
√%OAT	**.937**	**.956**	.262	.199	.088	−.157
%PHD	**−.917**	−.328	.170	−.015	.043	**.890**
%PSAP	**−.922**	**−.871**	.051	.091	−.099	.241
CAE	−.015	.236	**.558**	**.936**	**.739**	−.056
TAE	.485	**.649**	.483	**.728**	**.668**	−.127
Rears	−.115	−.284	−.111	**.696**	**.849**	**−.534**
THD	**.583**	**.947**	**.615**	.124	.254	.116
TSAP	.055	−.192	**.679**	**.763**	.236	.001
%HUB	−.229	.433	**.745**	**.748**	−.169	.102
% variance	41.1	43.6	20.5	31.0	19.0	12.3
Percentage of total variance	Male=80.7% Female=86.9%					
Sampling adequacy	Male .65 Female .65					

Factor loadings for both male and female rats (collapsed across age) from data set 2 are listed, with loadings higher than 0.5 (or less than −0.5) enlarged and boldfaced for emphasis. Abbreviations for behaviors analyzed are described in Table 1.

Table 6
Factor loadings of elevated plus-maze behaviors of both male and female rats from data set 3

Data set 3						
Behavior	Factor 1		Factor 2		Factor 3	
	Male	Female	Male	Female	Male	Female
%OAE	**.871**	**.921**	.129	−.092	.253	−.074
√%OAT	**.865**	**.933**	.376	.212	.131	.209
%PHD	**−.755**	**−.622**	−.208	−.077	.183	−.161
%PSAP	**−.840**	**−.760**	.033	−.069	.062	.197
CAE	.133	−.034	**.944**	**.974**	.026	.168
TAE	.465	.392	**.846**	**.896**	.093	.103
Rears	−.225	−.434	.325	.388	**.763**	**.659**
THD	**.822**	**.774**	.390	.451	.176	.228
TSAP	.358	.130	−.175	−.073	**.713**	**.914**
%HUB	**.544**	.152	.370	.276	.070	**.633**
% variance	41.7	36.7	22.3	22.5	12.5	18.8
Percentage of total variance	Male=76.5% Female=77.9%					
Sampling adequacy	Male .60 Female .58					

Factor loadings for both male and female rats (collapsed across age) from data set 3 are listed, with loadings higher than 0.5 (or less than −0.5) enlarged and boldfaced for emphasis. Abbreviations for behaviors analyzed are described in Table 1.

that factor 3 was more often an activity-like factor in males. For the female animals, factor 3 was less clear across studies, with no one behavior loading consistently on this factor across all three data sets. Although not a consistent trend, risk-assessment-like behaviors seemed to be the most probable component for factor 3 in female animals, with TSAP and rears loading strongly in 2 of 3 data sets, whereas %PHD and %HUB loaded highly once across the three data sets.

4. Discussion

In these factor analyses used to characterize EPM behavior of both adolescent and adult animals, the primary factor (accounting for the greatest percentage of the total variance) was largely and reliably composed of behavioral measures thought to reflect anxiety. Additional components emerging in these analyses of the EPM consisted of a second factor, for which behaviors traditionally argued to reflect activity (Cruz et al., 1994; Rodgers and Johnson, 1995) and/or protected exploration (Fernandes and File, 1996; Wall and Messier, 2000) loaded highly, and a third factor that primarily demonstrated high loadings for behaviors thought to reflect risk-assessment in the EPM (Cruz et al., 1994; Fernandes and File, 1996; Rodgers and Johnson, 1995). When subsequent analyses were conducted to compare male and female animals, again anxiety-like behavioral measures loaded strongly on the primary emerging component of EPM behavior. However, within the analyses separated by sex, factors 2 and 3 were somewhat more variable and most often consisted of a combination of activity and risk-assessment behaviors, especially in males.

Similar to these results, previous studies which utilized factor analysis to examine the EPM test have also demonstrated that, at least in adult male rats, anxiety measures consistently and highly loaded on the primary component of behavior (Cruz et al., 1994; Fernandes and File, 1996; Wall and Messier, 2000). In these experiments, the classic anxiety measures, %OAT and %OAE, were highly and inversely related to what have been argued to be more ethologically relevant anxiety behaviors, such as %PHD and %PSAP (Rodgers and Dalvi, 1997; Rodgers and Johnson, 1995), with the present results replicating this pattern. However, to our knowledge, these data are the first to provide evidence that, as is the case for adults, the principal EPM behavioral component in adolescent animals across a variety of different experimental conditions is also anxiety. These results are important, since popular behavioral models of anxiety were originally developed for use with adults, and hence before these tests of anxiety can be used to compare modulators of anxiety in adolescence and adulthood, it is important to verify that they in fact measure the same behavioral aspects in adolescents as in adults.

Prior research examining sex-related differences in EPM behavior has focused mostly on quantitative differences between males and females in terms of exhibited EPM behavioral measures. In fact, few studies have employed factor analysis to compare potential qualitative sex differences in underlying components of the behavioral repertoire in the EPM. Fernandes et al. (1999) did analyze the principle components of EPM behavior in both male and female adult rats and observed a sex difference in the components that emerged. In males, a 2-factor model emerged with the classic profile of anxiety measures loading strongly on the first factor and activity measures on the second. In contrast, female animals demonstrated the opposite pattern: the primary behavioral component was activity and the second, anxiety. The results from our sex analyses are dissimilar to these, in that factor 1 was comprised of anxiety measures for both males and females in our data sets. Interestingly, within our present 3-factor solution, a trend was observed wherein female animals more commonly demonstrated high factor loadings for activity measures on factor 2 and risk-assessment behaviors on factor 3, whereas males tended to demonstrate the opposite pattern. Thus, even within our 3-factor solution, activity measures accounted for more of the total variance in female EPM behavior compared to males. The contrasting results between this and the Fernandes et al. (1999) study could be due to several methodological differences between the two laboratories. These differences include testing different strains of rats (Wistar rats were used in Fernandes et al., 1999); inclusion of more behavioral measures in the factor analyses within the present investigation; and testing of animals in the current study only in the EPM, whereas animals in Fernandes et al. (1999) were given a 5-min exposure to the hole board test immediately prior to EPM testing. Such methodological differences are important to consider when generalizing results across laboratories because several studies have shown the profound impact that testing procedures may have on subsequent EPM behavior (Doremus and Spear, in preparation; Doremus et al., 2004; Griebel et al., 1993; Hogg, 1996; Morato and Brandao, 1996; but see also Falter et al., 1992).

In terms of interlaboratory methodological differences, it is of particular importance to consider apparatus size when examining potential ontogenetic variations in behavior. Since

considerable size discrepancy exists between adolescent and adult animals, behavioral assays should be appropriately size-adjusted to account for these developmental differences. Specifically, the EPM test relies on the conflict between the desire to explore novel areas and the fear of open elevated spaces. In as much as the size of the platform contributes to the feeling of safety for an animal on the open arms, a larger platform relative to body size could allow for more exploration of the open arms in adolescents tested in an adult-sized apparatus. Separate apparatuses were used for each age group within this study, therefore greatly reducing the potential impact of body size differences on the results obtained. Yet, there are sizable differences between the relative body size of males and females by the time of adulthood, and this could have potentially impacted anxiety levels and behavior in the EPM for the adult female group. Indeed, factor analysis results were quite variable for factors 2 and 3 across the data sets in the present sex analyses, with variability due to smaller body size relative to the apparatus size in the adult female group compared to other age and sex groups a possible contributor to these inconsistencies. To our knowledge, no studies have used separately scaled EPM apparatuses for adult females versus males, although some researchers have attempted to examine sex differences in EPM behavior at slightly different ages when sizes are more comparable between the adult sexes (i.e. test females at an older age than males) (Johnston and File, 1991).

Taken together, the results of the present factor analyses demonstrate that behaviors thought to index anxiety form the primary underlying component of behavior for both adolescent and adult male and female rats. These results document the potential suitability of the EPM as a behavioral anxiety assay for rodents during adolescence and adulthood. However, caution is necessary when assessing the loading of other activity/risk assessment behaviors due to some inconsistencies across data sets. While these results are promising with regard to use of the EPM in adolescent animals, other data from our laboratory caution that the specific pretest circumstances used prior to the actual EPM test may exert a differential impact on behavior across age (Doremus et al., 2004). Thus, although the underlying components of EPM behaviors are similar in adolescents and adults, care is needed when designing experiments and drawing conclusions while using the EPM to examine ontogeny of anxiety related behaviors.

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