Temperamental traits in mice (I): Factor structure

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Abstract

The present research develops a murine model of the temperamental traits activity, anxiety and novelty seeking.
A sample of 140 outbred male mice was assessed in the plus maze (EPM), low frightening open field (LFOF), holeboard (HB) and T maze (TM). An exploratory factor analysis (EFA) was applied to sub-sample 1 (N = 70), and a confirmatory factor analysis (CFA) was applied to sub-sample 2 (N = 70).
EFA showed a three-factor solution: activity in LFOF and HB loaded in the first factor; inhibitory behaviours in the EPM loaded in a second factor; and approach behaviour to novel environment in TM loaded in a third factor. CFA analysis confirmed this structure.
We have described three replicable factors in mice that could be considered homologous to human personality traits activity, anxiety and novelty seeking.

Keywords: Personality; Temperament; Novelty seeking; Activity; Anxiety; Outbred mice; Exploratory factor analysis; Confirmatory factor analysis

1. Introduction

Nowadays it has been demonstrated that genetic factors contribute to individual differences in human personality (Plomin, 1990). According to quantitative genetic studies, heritability in...
personality traits is estimated between 30% and 60% (Bouchard & Loehlin, 2001). Molecular genetic studies have begun to find several genes and chromosomal loci that are related to human personality traits, such as novelty seeking or anxiety (Lesch, 2003; Reif & Lesch, 2003). Genes influence personality through heritable biological systems (Eysenck & Eysenck, 1985; Zuckerman, 1991), and these biological systems appear to be located in primitive brain areas which are at least partially conserved between species (i.e. nucleus accumbens and amygdala; Depue & Collins, 1999; Gray & McNaughton, 2000; Lesch, 2003). From an evolutionary perspective it can therefore be hypothesised that the homologous brain systems in other animal species may regulate behavioural traits which are homologous to human personality traits (Bouchard & Loehlin, 2001; Cloninger, 1994; Gosling, 2001).

Gosling (2001) has described those personality traits which have been more frequently studied in nonhuman animal species. A small number of these traits appear to be common to most nonhuman mammals, although their identification depends on the particular animal species studied, and the behavioural domain sampled. These core traits include emotionality/fearfulness, exploration/novelty seeking, activity, aggression and sociability. Similar temperamental traits have also been described in humans (Zuckerman, Kuhlman, & Camac, 1988, 1991), and they have been related to the main personality models, such as the Eysenck, Gray, Cloninger or Costa and McCrae models (Zuckerman, 1999; Zuckerman & Cloninger, 1996; Zuckerman, Kuhlman, Joireman, Teta, & Kraft, 1993). Thus, the study of temperamental traits in animals seems a useful strategy in determining the basic dimensions of personality (Eysenck, 1991; Zuckerman, 1992).

Although a large number of studies have been conducted in rats and mice, these studies have usually focused on specific traits, and only a limited number of studies have attempted to investigate the latent structure of traits in rodents (Gosling & John, 1999). Some rodent studies have applied a factor analysis to behaviours in one specific apparatus, typically the open field (OF; e.g., Crusio, 2001; García-Sevilla, 1984; Ortet & Ibáñez, 1999) or the elevated plus maze (EPM; see Wall & Messier, 2001). However, the use of a battery of tests is a better option because it also allows one to study basic characteristics of temperamental traits, such as the stability and consistency (e.g., Belzung & LePape, 1995; Ramos, Berton, Mormède, & Chaouloff, 1997; Ramos, Mellerin, Mormède, & Chaouloff, 1998). One of the most robust findings in these factorial studies is the presence of two consistent underlying traits: an anxiety trait, typically inhibitory behaviour when faced with natural aversive stimuli; and an activity trait, typically horizontal (i.e., ambulation) and vertical (i.e., rearing) movements (see Ramos & Mormède, 1998). When exploration behaviour tests of novel objects or environments have also been included, an independent factor apart from anxiety or activity has been found and named exploration (e.g., Fernandes & File, 1996; Fernandes, González, Wilson, & File, 1999; Lister, 1987) or neophilia (Belzung & LePape, 1995).

However, methodological and conceptual limitations in some of these studies, especially those including exploration tests (e.g., inadequate ratio number variables/subjects for factor analysis, lack of rotation, or questionable number of factors extracted), recommend a better methodological approach and a more systematic exploration of battery of tests within a personality framework. Thus, the objective of the present research is to study behaviour patterns that may be homologous to human activity, anxiety and novelty seeking traits in a genetically heterogeneous outbred strain of male mice.

With this in mind, we will use some of the most used and valid tests to assess activity, anxiety and novelty seeking in rodents. The most commonly used observational method for measuring
activity in rodents is the assessment of ambulation in the OF (Kelley, 1993), especially the low frightening version (low levels of light and sound; LFOF; see García-Sevilla, 1984). There are several ethological measures proposed to assess anxiety in rodents (see Ramos & Mormède, 1998), although the elevated plus maze (EPM) is most widely used (Hogg, 1996). This apparatus measures inhibitory behaviour or innate passive avoidance from natural aversive stimuli, such as open space and height (Graeff, Netto, & Zangrossi, 1998). Another widely used index in the assessment of anxiety is defecation in the OF (e.g., Crusio, 2001; García-Sevilla, 1984). Finally, different apparatus have been designed to assess exploration or novelty seeking (Bardo, Donohew, & Harrington, 1996). One of the most widely used free-choice novelty tests is the holeboard (HB), a test developed to measure the exploration of holes (head-dipping; File, 2001; File & Wardill, 1975). Another free-choice novelty test that has also been used and validated in the assessment of novelty seeking is the T-maze, which measures the approach behaviour to a novel environment (Dellu, Mayo, Piazza, Le Moal, & Simon, 1993).

Thus, we will examine the factor structure of different behaviours, assessed in at least two different apparatus, used to reflect independent activity, anxiety and novelty seeking traits using the exploratory factor analysis (EFA). Furthermore, we will replicate the factor structure found with the EFA by using the confirmatory factor analysis (CFA) in an independent sample. Specifically, we will assess ambulation behaviours in LFOF and in HB as indexes of an underlying activity trait. We will also assess behavioural inhibition in the EPM and defecation in the LFOF as indexes of an underlying anxiety trait. Finally, we will go on to assess approach behaviour to holes in the HB and to novel places in the TM as an index of an underlying novelty seeking trait.

2. Method

2.1. Animals

A total sample of 140 male mice of the outbred strain Swiss Albino was obtained from Interfauna Ibérica, S.A., Barcelona, Spain (Sprague Dawley, Co.). Animals were 5 weeks old at the start of testing and were housed 5 per cage with freely available water and food. All mice were maintained at a room temperature of 22 °C with controlled humidity, where a 12 h light–dark schedule was in effect. Testing took place during the light phase of the cycle.

2.2. Apparatus

The apparatus have been self-made according to the following references: Elevated plus maze (Lister, 1987); Holeboard (File & Wardill, 1975); Low-frightening open field (Ortet & Ibáñez, 1999). Furthermore, we have adapted for mice the T maze described for rats in Dellu et al. (1993).

Elevated plus maze (EPM). The EPM was made of black plastic and consisted of two open arms $30 \times 5 \times 5$ cm and two enclosed arms $30 \times 5 \times 15$ cm. The arms extended from a central platform $5 \times 5$. The apparatus was raised 40 cm above the floor.

Holeboard (HB). The HB was a clear plastic box $40 \times 40 \times 30$ cm with four holes 3 cm in diameter equally spaced in the floor. The floor was divided into 16 equal squares.
T-maze (TM). The TM was made of black plastic and consisted of three equal arms 25 × 8 × 20 cm which extended from a central platform 8 × 8 cm. Each arm had a guillotine door and different coloured stimuli on its walls.

Low-frightening open field (LFOF). The LFOF was a clear plastic box 50 × 50 × 40 cm and its floor was divided into 25 equal squares.

2.3. Procedure

One week after arrival, behavioural testing was carried out three hours after the light cycle, and it began in a soundproofed room under dim illumination. The subjects order was counterbalanced daily. The order administration of the apparatus was also counterbalanced, apart from the EPM that was always used on the first day of the experiment. Each animal was assessed once in subsequent tests:

Day 1. EPM. The following measurements were taken for 5 min (see File & Wardill, 1975):
– Time spent in open arms (time OA EPM)
– Entries made into open arms (entries OA EPM)

Day 2– 4 (counterbalanced). HB. The following measurements were taken for 5 min (see Lister, 1987):
– Number of squares crossed (activity HB)
– Number of head-dipping (head-dipping HB)

LFOF. The following measurements were taken for 5 min (see Ortet & Ibáñez, 1999):
– Number of inner and outer squares crossed (activity LFOF)
– Number of boli deposited (defecation LFOF)
– Number of rearing (rearing LFOF)

TM. An experiment consisted of two trials separated by a 30 min interval. In the first trial, one arm of the TM was closed with a guillotine door. The mice were allowed to visit the two arms for 10 min. During the second trial, animals had free access to the three arms, and were allowed to explore the maze for 5 min. Two behaviours were measured in the second trial (see Dellu et al., 1993):

– Time spent in novel arm (time NA TM)
– Entries made into novel arm (entries NA TM)

2.4. Data analyses

We divided the total sample into two equal sub-samples of seventy mice each (n1 = 70; n2 = 70). Descriptive statistics and Pearson correlations were calculated for each sub-sample. The t-test for independent samples was applied in order to study significant mean differences between sub-samples 1 and 2. A principal axis factor analysis with oblimin rotation was carried out in sub-sample 1. The criteria for the number of factors extracted were the scree-test, eigen-value
greater than one, and conceptual considerations discussed in the introduction section. The CFA using AMOS 4.0 statistical software (Arbuckle, 1999) was applied in sub-sample 2.

3. Results

Table 1 details descriptive statistics. The t-test mean comparison showed no differences between sub-samples 1 and 2 (data not shown). Thus, both samples were comparable as far as their behavioural characteristics are concerned. Table 2 presents the correlations among the assessed variables in sub-samples 1 and 2.

Prior EFA analyses were conducted and included all variables (data not shown). According to scree-test and eigen-value criteria, three factors were extracted. Defecation LFOF and head-dipping HB showed unsatisfactory commonality values (lower than 0.20), suggesting that these factors do not adequately explain the variance of defecation and head-dipping. Furthermore, defecation LFOF and head-dipping HB presented low and unexpected loadings (i.e., defecation loaded inversely in factor 2, meanwhile head-dipping loaded only marginally in factor 1). Thus, defecation and head-dipping were not included in the final EFA. Fig. 1 shows the scree plot, and Table 3 shows the obtained oblique rotated factor solution. The factor structure accounted for 80.51% of the

Table 1
Descriptives (mean, standard deviation, minimum and maximum values) for the behaviours assessed in low frightening open field (LFOF), elevated plus maze (EPM), T maze (TM) and holeboard (HB) in sub-sample 1 (n = 70) and sub-sample 2 (n = 70)

<table>
<thead>
<tr>
<th></th>
<th>Sub-sample</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity LFOF</td>
<td>1</td>
<td>163.9</td>
<td>39.7</td>
<td>81</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>153.9</td>
<td>48.1</td>
<td>19</td>
<td>251</td>
</tr>
<tr>
<td>Rearing LFOF</td>
<td>1</td>
<td>32.5</td>
<td>11.9</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>28.1</td>
<td>15.1</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>Defecation LFOF</td>
<td>1</td>
<td>3.1</td>
<td>1.8</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.3</td>
<td>2.3</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Activity HB</td>
<td>1</td>
<td>113.4</td>
<td>32.9</td>
<td>48</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>115.3</td>
<td>30.5</td>
<td>58</td>
<td>192</td>
</tr>
<tr>
<td>Head-dipping HB</td>
<td>1</td>
<td>13.8</td>
<td>4.7</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.2</td>
<td>3.8</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>Entries OA EPM</td>
<td>1</td>
<td>3.1</td>
<td>1.8</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.3</td>
<td>2.3</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Time OA EPM</td>
<td>1</td>
<td>22.8</td>
<td>18.8</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.8</td>
<td>18.1</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>Entries NA TM</td>
<td>1</td>
<td>10.1</td>
<td>2.5</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.6</td>
<td>2.4</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Time NA TM</td>
<td>1</td>
<td>104.3</td>
<td>28.2</td>
<td>44</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>99.5</td>
<td>28.8</td>
<td>45</td>
<td>187</td>
</tr>
</tbody>
</table>
total variance: The first factor accounted for 36.68% of the variance, the second accounted for 27.15% while the third represented 16.68% of the total variance. Finally, factor intercorrelations
were non-significant, although factors 1 and 2 presented a tendency of 0.20, and factors 1 and 3 presented a tendency of 0.25.

Based on the obtained EFA solution, we tested the goodness-of-fit for two models using CFA. The initial model hypothesised that: (a) activity LFOF and activity HB load in the activity factor; (b) entries and time OA EPM load in the anxiety factor; (c) entries and time NA TM load in the novelty seeking factor; and (d) the factor interrelations were freely estimated. Based on modification indexes suggested by the AMOS program in order to increase the fit to the data, we tested a second model that included: (a) entries NA TM also load in activity factor; and (b) error covariance between activity HB and time OA EPM. This model was taken as our final model.

The fit indexes of initial and final models are summarised in Table 4. Although the initial model presented acceptable values in most approximated fit indexes, the $X^2/p$ value is lower than 0.05 and the RMSEA (root mean squared error of approximation) index is greater than 0.05. The final model showed adequate goodness of fit indexes and it is illustrated in Fig. 2 (estimated parameter for error covariance between activity HB and time OA EPM: $-0.34$, $p = 0.013$; data not shown).

4. Discussion

The study of human homologous behavioural traits in other animal species constitutes an undeveloped but useful and necessary line in personality research (Gosling, 2001). Accordingly
when referring to animal models of personality, Flint (2003) pointed out that “Perhaps the most critical skill that will be needed for postgenomic behavioural genetics will be that of the experimental psychologist, in devising the most appropriate behavioural tests” (Flint, 2003, p. 437). This is the main aim of the present research.

The oblique rotated factor solution obtained in EFA shows three independent factors. The first factor comprises horizontal movements (i.e., ambulation) in different apparatus, such as LFOF and HB, and also vertical movements (i.e., rearing) in LFOF. It was therefore labelled as activity. The second factor included an inhibitory behaviour to the open arms of the EPM, so the factor was labelled (low) anxiety. The third factor referred to the approach behaviours to novel environment in the TM, and this factor was labelled novelty seeking.

The findings obtained with the EFA were replicated by means of a CFA in an independent sample. Furthermore, CFA also revealed that entries NA TM had a secondary loading in the activity factor, suggesting that both activity and novelty seeking factors would influence ambulation behaviour. Thus CFA showed a robust three-factor structure where the behaviours characterising the activity, anxiety and novelty seeking factors were the same as those in the EFA. CFA has been seldom used in animal studies, but it has proved most useful in interpreting the factor analysis in a parsimonious way. For example, several studies have found between 2 and 6 factors using EFA in the EPM (see Wall & Messier, 2001). However Wall and Messier (2000) have demonstrated that only the activity and anxiety factors would adequately fit the data when CFA was applied.

Thus, it has been shown that it is possible to describe a robust three-factor structure in outbred male mice which underlies several behaviours in our battery of apparatus. Ambulation and rearing in several apparatus are influenced by a common underlying activity trait. This result is in accordance with other studies which found that these two variables are partially regulated by the same genetic background (Gershenfeld et al., 1997; Trullas & Skolnick, 1993). The anxiety factor comprises (in negative) time and entries OA EPM, measures that have been extendedly interpreted as indexes of anxiety (Hogg, 1996; Ramos & Mormède, 1998; Wall & Messier, 2001). Finally, time and entries NA TM formed an independent novelty seeking factor, which is in accordance with several proposals (Bardo et al., 1996).

Contrary to what we expected, we found that both the exploration of holes in the HB and exploration of novel places are not influenced by the same underlying trait. Belzung and LePape (1995) found similar results, and Escorihuela et al. (1999) described that head-dipping may reflect a novelty seeking trait, especially when novel objects are included in the holes of the HB, but not when using holes without novel objects. In addition, and according to our findings, it has been suggested that defecation presents certain doubts in the measure of anxiety, at least when LFOF is used (Crusio, 2001; Ramos & Mormède, 1998). Probably, head-dipping and defecation may be valid indexes of novelty seeking and anxiety respectively, but only in certain experimental conditions, e.g., when novelty and anxiety are increased via the environment (e.g., placing objects for sensation seeking and increasing light and sound levels for anxiety) or via genes (e.g., using high novelty seeking and high anxiety strains).

Factorial studies in rats and mice have consistently supported the presence of at least two independent and consistent traits, activity and anxiety: activity typically characterised by ambulation in low frightening environments; anxiety typically characterised by inhibitory behaviour facing natural threats (see Ramos & Mormède, 1998). Furthermore, it has been demonstrated that the activity and anxiety factors are influenced by independent genetic background. At the same time,
the cross-apparatus consistency found for activity is attributed to common genetic factors, just as in anxiety (Ramos et al., 1997; Ramos et al., 1998).

Other factorial studies have found a third exploration factor with head-dipping in the HB acting as a marker (Fernandes & File, 1996; Lister, 1987), making its comparison to the present study difficult since head-dipping did not seem an adequate index of novelty seeking. More comparable is the factorial study by Belzung and LePape (1995). They extracted two unrotated factors: the first was labelled neophilia, and it comprised approach behaviours to novel objects and places in different apparatus; the second included different behaviours from both the HB and EPM. It has been suggested that this second factor could be divided into two activity and anxiety factors (Ramos & Mormède, 1998), and we propose that the neophilia factor may be interpreted as a novelty seeking factor from a personality perspective (Bardo et al., 1996; Cloninger, 1998).

In this sense, a quantitative trait loci study carried out by Turri, Datta, DeFries, Henderson, and Flint (2001) described how three different genetic loci influenced activity, avoidance behaviour and exploration. Furthermore, this study also suggests that emotional elimination and avoidance behaviour are genetically independent variables. Thus, as in the present research, different studies confirm the presence of at least three genetically- and biologically-based systems which underlie three consistent temperament factors: activity, anxiety and exploration/novelty seeking in rodents.

Activity factor refers to vigour and unspecific activity level, a definition that fits reasonably well with the human activity trait (Zuckerman et al., 1988, Zuckerman, Kuhlman, Thornquist, & Kiers, 1991). Anxiety factor refers to inhibitory behaviour elicited by threats, and also fits well with the human anxiety construct proposed by Gray (Gray & McNaughton, 2000) and Cloninger (1998). Novelty seeking refers to activation and approach behaviour to novelty, a definition that fits well with the human trait novelty seeking (Bardo et al., 1996; Cloninger, 1998; Zuckerman, 1991).

The factor structure found in mice also paralleled that found in humans. Personality research has proposed the existence of at least three basic personality dimensions referring to anxiety-related traits (e.g., anxiety, harm avoidance, neuroticism), novelty seeking-related traits (e.g., novelty seeking, sensation seeking, impulsivity) and activity-related traits (e.g., activity, extraversion, reward dependence) (see Bouchard & Loehlin, 2001). Zuckerman et al. (1988, 1991) have found that the factor structure of different scales, which were developed to assess basic human personality traits within a psychobiological framework, led to five factors: Impulsive Unsocialized Sensation Seeking (ImpUSS), Neuroticism-Anxiety (N-Anx), Activity (Act), Sociability (Sy) and Aggression-Hostility (Agg-Host). Novelty seeking would form part of ImpUSS (Zuckerman & Cloninger, 1996), whereas N-Anx and Act are conceptually equivalent to the anxiety and activity factors, as described in the present study.

Thus, according to factor content and factor structure, we consider that the proposed battery of tests may be preliminarily used in the research of murine temperamental traits that are homologous to human personality traits of activity, anxiety and novelty seeking. However, our results are obtained in males, so replication studies in female samples are needed. Furthermore, the relative stability and consistency over time and apparatus for the anxiety and novelty seeking factors have to be investigated further. Stability and consistency are core characteristics of temperamental and personality traits (Eysenck & Eysenck, 1985; Zuckerman, 1991). Initially, we included different behaviours in at least two different apparatus in order to extract stable and consistent underlying factors. Under our experimental conditions however, neither head-dipping nor defecation were
related to other novelty seeking and anxiety behaviours, respectively. Thus, the extracted anxiety and novelty seeking factors refer to the behaviours in only one test, so these factors may reflect underlying traits, or alternatively, may reflect apparatus specificity. Further studies are required to explore the cross-apparatus consistency of the described factors, and thus their temperamental nature.

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References


