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Genetic differences in the elevated plus-maze persist after first exposure of inbred rats to the test apparatus

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Abstract

The elevated plus-maze (EPM) is an anxiety model thought to assess different types of emotional states depending on whether or not the animals have been previously exposed to the test apparatus. Accordingly, benzodiazepine-treated rodents generally differ from controls in the first but not in the second EPM trial. Inbred Lewis and SHR rats of both sexes ($N = 10$) were submitted twice (test and retest) to the EPM with a 24 h interval between trials. Overall strain differences (Lewis < SHR) were observed in both males and females concerning anxiety-related measures (time spent and percent of entries in the open arms) regardless of previous maze experience. Moreover, prior exposure to the test apparatus produced an overall decrease in the approach towards the open arms in both strains and sexes. The fact that genetic differences did not diminish or disappear in the second trial, suggests that test and retest in the EPM are likely to share some common emotional components and that differences between naïve LEW and SHR rats are not similar to those observed between control and benzodiazepine-treated animals.

Keywords: Anxiety; Elevated plus-maze; Lewis; Previous exposure; SHR

1. Introduction

The elevated plus-maze (EPM) is one of the most widely used animal models of anxiety and was developed based on the observation that rats avoid open elevated alleys and on the assumption that this avoidance is generated by fear (Montgomery, 1955). An extensive study by Pellow et al. (1985) validated this test through the use of behavioral, physiological and pharmacological approaches. When exposed to the EPM for the first time, a naïve rat or mouse will display signs of conflict, avoidance and escape, with avoidance of the open arms being decreased by classical anxiolytic drugs (i.e., benzodiazepines) and increased by anxiogenic substances (Handley and McBlane, 1993; Pellow et al., 1985). However, the response to anxiolytic drugs has been shown to diminish or even disappear when the animals are previously exposed for one

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single trial to the same test apparatus, a phenomenon known as "one-trial tolerance" (File, 1990). This phenomenon has been described not only for benzodiazepines (File et al., 1998, 1999; File and Zangrossi, 1993; Rodgers et al., 1992; Treit et al., 1993) but also for ethanol, phenobarbital and NMDA/glycine-B receptor ligands (Bertoglio and Carobrez, 2002a, 2002b, 2003).

Several studies have suggested that the type of emotion experienced by rodents in the EPM changes from the first to the second trial, but the exact psychological significance of this change remains unclear (File et al., 1993; Holmes and Rodgers, 1998; Rodgers et al., 1992). Although different pharmacological approaches have been used in the attempt to infer the type of fear/anxiety involved in the first and second trials of the EPM, to our knowledge, this matter has never been analyzed from a strain-comparison perspective. Yet, if rodent strains with contrasting emotional profiles were different from each other in the first but not in the second trial of the EPM, this would provide further support to the hypothesis that these two testing conditions asses different forms of emotional reaction.

The inbred rat strains Lewis (LEW) and spontaneously hypertensive rats (SHR) display contrasting behaviors in a variety of tests thought to be related to anxiety/emotionality and therefore have been used as a genetic animal model for the study of anxiety. They have been characterized in tests such as the open field, EPM, black/white box, social interaction and repeated defeat procedure (Berton et al., 1998; Ramos et al., 1997, 1998, 2002). In most but not all of these tests, LEW rats appeared to be more fearful than their SHR counterparts. However, differently from other pairs of rodent strains used as genetic models of emotionality, LEW and SHR rats display equal levels of locomotor activity (Ramos and Mormède, 1998). A molecular study using these two strains allowed the mapping of two suggestive QTL (quantitative trait loci), affecting the percent of open arm entries and the number of closed arm entries in the EPM, on rat chromosomes 6 and 7, respectively (Ramos et al., 1999). The aim of the present study was thus to compare LEW and SHR rats in both the first and second trials of the EPM in order to verify whether their genetic differences in anxiety-related behaviors would persist following prior exposure to this test.

2. Material and methods

2.1. Animals

The colonies of LEW and SHR rats used in this study were maintained in our laboratory under a system of brother–sister mating as it is generally recommended for inbred rodent strains (ILAR, 1992). The animals were weaned and separated by sex at 4 weeks of age and, thereafter, were kept in collective plastic cages (five rats/cage) with food and water available ad libitum under a 12L:12D cycle (lights on at 07:00 h) at $22 \pm 2\,^{\circ}$C. A total of 40 naive rats (10/strain/sex) were used in this study. At 8–9 weeks of age each rat was submitted twice to the EPM (a test and a retest) with an interval of 24 h between trials, which were always carried out between 14:00 and 17:00 h. In each testing session, LEW and SHR rats were tested in alternation but males were tested and retested two days before females. The present experiments were in accordance with the local regulations for the ethical use of animals in research (CEUA/UFSC) and covered by the valid permission no. 23080.002412/2001-26.

2.2. Elevated plus-maze (EPM)

The apparatus was made of wood covered with a layer of black formica and had four elevated arms (52 cm from the floor) 50 cm long and 10 cm wide. The arms were arranged in a cross-like disposition, with two opposite arms being enclosed (by 40 cm high walls) and two being open, having at their intersection a central platform (10 cm × 13.5 cm) which gave access to any of the four arms. The open arms were surrounded by a raised ledge (1 mm thick and 5 mm high) to avoid rats falling off the arms. The central platform was under 70 lx of illumination. Each rat was placed in the central platform facing an open arm and the following behaviors were registered for 5 min: the number of entries and the time spent (with all four paws) inside each type of arm and the percentage of open-arm entries in relation to the total arm entries. The behavior of each animal was recorded by a video camera positioned above the maze. The apparatus was cleaned with a wet sponge and paper towel between rats.

2.3. Statistics

The results were analyzed, separately for each sex, by a two-way ANOVA with trials (test and retest) being treated as repeated measures. In cases where significant interactions between factors (strain and trial) were found, the post hoc test LSD was used to compare the means of the groups. All analyses were carried out with the software Statistica (Statsoft France, 1996).

3. Results

The results concerning anxiety-related measures are shown in Fig. 1. For males, there was a general strain effect only for the time spent in the open arms (SHR > LEW; $F = 10.7$ and $p < 0.01$) but there was a general effect of previous exposure (test/retest) for both the time spent (test > retest; $F = 9.9$ and $p < 0.01$) and percent of entries in the open arms (test > retest; $F = 11.6$ and $p < 0.01$). For females, there was a general effect of strain for both the time spent (SHR > LEW; $F = 48.7$ and $p < 0.01$) and percent of entries in the open arms (SHR > LEW; $F = 21.0$ and $p < 0.01$). Similarly, a general effect of previous exposure was seen in the time spent (test > retest; $F = 14.6$ and $p < 0.01$) and percent of entries in the open arms (test > retest; $F = 14.2$ and $p < 0.01$).

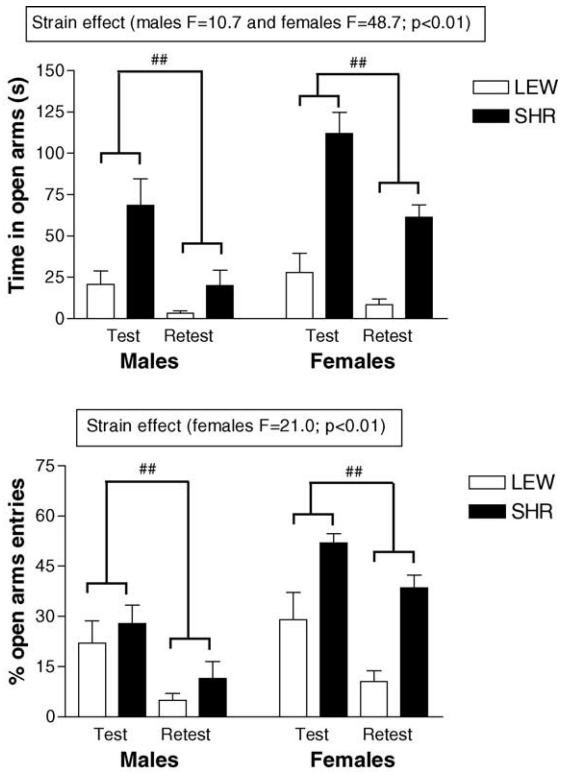

Fig. 1. Time spent and percent of entries in the open arms of the elevated plus maze of LEW and SHR male and female rats during their first (test) or second (retest) exposure. (##) Represents general previous-exposure effects (ANOVA, $p < 0.01$) in either males or females. General strain effects (ANOVA) for each sex across trials are shown in the box.

The results of locomotion-related measures are shown in Fig. 2. For males, there were interactions

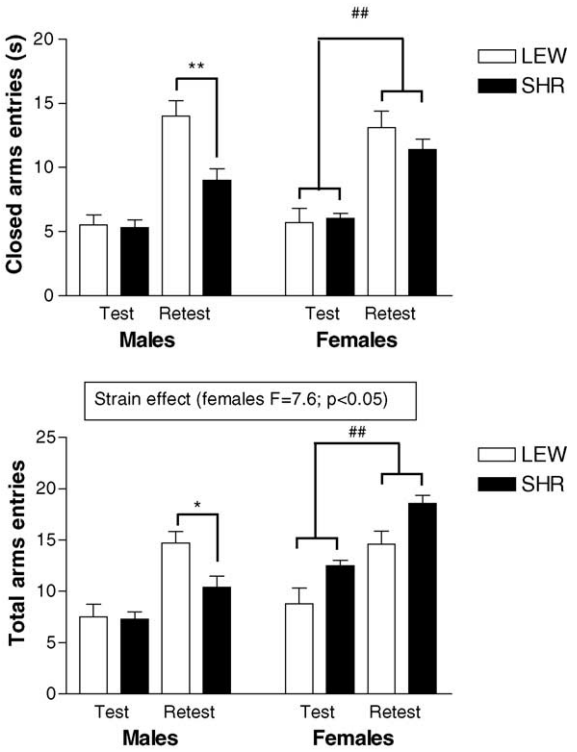

Fig. 2. Closed and total arms entries in the elevated plus maze of LEW and SHR male and female rats during their first (test) or second (retest) exposure. (##) Represents general previous-exposure effect (ANOVA, $p < 0.01$) in females. General strain effect (ANOVA) for females across trials are shown in the box. In males, there were significant interactions (ANOVA, $p < 0.05$) between strain and previous exposure for the number of closed and total arms entries, with LEW and SHR rats differing from each other only during the second exposure. These post hoc differences are represented by $^{*}p < 0.05$ and $^{**}p < 0.01$, respectively.

between strain and previous exposure for the number of closed ($F = 7.3$ and $p < 0.05$) and total arm entries ($F = 5.2$ and $p < 0.05$), with LEW and SHR rats differing from each other only during the retest. For females, there was a general effect of strain only in the total number of arm entries (SHR > LEW; $F = 7.6$ and $p < 0.05$) but a general effect of previous exposure was seen for both closed (retest > test; $F = 118.2$ and $p < 0.01$) and total (retest > test; $F = 71.4$ and $p < 0.01$) arm entries.

4. Discussion

In the present study, the LEW and SHR inbred rat strains, which are known to differ in several behavioral tests of anxiety/emotionality (Ramos et al., 1997, 1998, 2002), were submitted to the EPM with or without a previous exposure to the test, two conditions that have been suggested to correspond to acquired phobia and generalized anxiety disorder (GAD), respectively (File and Zangrossi, 1993). Two main arguments have been used to support such a correspondence: (i) benzodiazepines, which are effective against GAD but not against phobias, have anxiolytic-like effects in the first but not in the second trial of the EPM; and (ii) when the first trial is longer than usual (i.e. 10 min instead of 5 min), the phenomenon of one-trial tolerance disappears, thus providing an analogy with phobic states, that diminish with exposure to the phobic situation (File et al., 1993). Other authors, however, whereas recognizing that there is a qualitative shift in the type of emotionality experienced during the first and second trials, consider that the psychological significance of this change is unclear (Holmes and Rodgers, 1998). The present results reinforce such a cautious interpretation.

In the two successive trials, overall differences between rat strains were observed for both indices of anxiety from the EPM, namely the time spent and the percent of entries in the open arms (Pellow et al., 1985). Whereas in females both these effects were significant, in males the strain differences reached statistical significance only for the time spent in the open arms. On the other hand, animals of both strains and sexes showed a significant decrease in the time spent and percent of entries in the open arms when retested 24 h after their first exposition, which is in agreement with previous reports (Bertoglio and Carobrez, 2000, 2002c; Rodgers et al., 1992). It is not known which specific stimulus in the test induces a higher avoidance of the open arms in the second trial, but there is experimental evidence pointing to the openness of the arms rather than their novelty or height (Aguilar et al., 2002; Pellow et al., 1985; Treit et al., 1993).

In the present study, a significant increase in the closed and total arm entries—two putative measures of locomotor activity (see Ramos and Mormède, 1998)—was detected for females during the retest (with a non-significant trend in the same direction being observed in males), thus suggesting that locomotion levels increase during the second trial, possibly due to a higher familiarity with the test apparatus. Interestingly, such changes were paralleled by an apparent increase in anxiety (i.e. lower approach to the open arms), thus suggesting that higher fearfulness does not result in lower locomotor activity in the closed arms. Several studies using principal component analyses applied to EPM behaviors showed that the approach towards the open arms (anxiety) and the number of entries in the closed arms (locomotion) in the first trial load on two different factors, being thus independent for the most part (Cruz et al., 1994; Ramos et al., 1997; Trullas and Skolnick, 1993). Whether these measures are also independent in the second EPM trial remains to be investigated.

No interaction between strain and previous experience was observed in the time spent in the open arms and in the percent of open arm entries, thus indicating that the anxiety-related differences between LEW and SHR rats in the EPM persist after one exposure to the test apparatus. The strain differences observed in the first trial of the present study are in agreement with our previous reports (Ramos et al., 1997, 1998, 2002), which suggest that LEW rats of both sexes are more anxious-like than their SHR counterparts. However, these two strains had never been compared in a retest in the EPM. The possibility that these strains differ in both GAD and phobic state models (which would explain why they differ in both first and second trials) is not supported by parallel studies from our laboratory, which failed to reveal significant differences between LEW and SHR rats in the cat odor test (unpublished data), another proposed model of phobia.

Previous pharmacological studies indicate that both LEW and SHR strains respond to the anxiolytic effects of benzodiazepines when submitted to the EPM for the first time, but LEW rats seem to require higher doses of diazepam before their anxious-like profile

is attenuated (Ramos et al., 1997; Takahashi et al., 2001). Further studies are necessary to investigate these strains' responses to benziodiazepines in the second trial of the EPM.

If the behavioral/emotional differences between naïve LEW and SHR rats were similar in nature to those typically observed between control and benzodiazepine-treated rodents, then one would expect that the strain differences seen in the first EPM trial would diminish or disappear in the retest. The lack of interaction between strain and trial observed herein, however, does not corroborate such a hypothesis. Benzodiazepine-treated rats and mice generally differ from controls in the first but not in the second plus-maze trial, whereas LEW and SHR rats were shown here to differ in both test situations. Thus, the present results suggest that mechanisms not acting through the GABA/benzodiazepine system should underlie, at least in part, the emotional differences observed between the LEW and SHR strains. Moreover, through a genetic approach, these results provided a link between the first and second trials of the EPM, suggesting that the emotional responses elicited by these two testing conditions may share common psychological components.

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