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## BRIEF COMMUNICATIONS

# Confinement to the Open Arm of the Elevated-Plus Maze as Anxiety Paradigm: Behavioral Validation

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Exposure to the open arm of the elevated-plus maze was used to assess the neurobiological correlates of anxiety in the high-anxiety-related behavior (HAB) and low-anxiety-related behavior (LAB) rat lines. The authors sought to determine whether this mild stressor could be considered a valuable anxiety test revealing specific behavioral differences. Behavioral parameters scored were submitted to a discriminant and factor analysis to investigate emotional parameters discriminating HAB and LAB rats. Principal component analysis showed that the HAB rats' behavior was driven by anxiety, whereas the LAB rats' behavior was mainly explained by locomotor activity. Moreover, the rats displayed behaviors that reflected distinct coping strategies confirming anxiogenic open arm effects and differential appraisals of the stressor dependent on the genetic predisposition to either hyper- or hypo-anxiety.

*Keywords:* open arm, trait anxiety, coping strategy, principal component analysis, high-anxiety-related behavior

Affective disorders such as anxiety and depression represent a major public health problem as life prevalence rates for experiencing any anxiety disorders have varied from 10.4% to 25.1% (Costa e Silva, 1998; Lecrubier, 2001). Within the last decades, two rat lines derived from the Wistar strain were selected and bred on the basis of their high-anxiety-related behavior (HAB) and low-anxiety-related behavior (LAB) in the elevated-plus maze (Landgraf & Wigger, 2002; Liebsch, Montkowski, Holsboer, & Landgraf, 1998). Various studies have clearly established that HAB rats can be regarded as a reliable model of innate anxiety (Landgraf & Wigger, 2002; Salomé et al., 2002). When exposed to the open arm of the elevated-plus maze, which is considered as a mild emotional stressor and described to induce higher fear-anxiety responses than an exposure to the closed arm (Pellow,

Chopin, File, & Briley, 1985), HABs rats have shown a hyper-reactivity of the hypothalamo-pituitary-adrenal axis (Landgraf, Wigger, Holsboer, & Neumann, 1999). Additionally, in this condition, in HAB rats, the release of hippocampal serotonin did not change contrary to LABs in which it increased (Keck et al., 2005; Umriukhin, Wigger, Singewald, & Landgraf, 2002), and HABs displayed the highest corticotropin releasing hormone receptor 2 binding in the ventromedial hypothalamus and central amygdala (Wigger et al., 2004). Furthermore, HAB rats have displayed a higher expression of Fos protein, a marker of cellular activation, in hypothalamic and limbic structures and a lower expression in the prefrontal cortex in comparison with LAB rats (Salomé et al., 2004). However, despite the extensive use of this mild stressor for neurobiological approaches, no study has precisely described the behavioral profiles of both lines during exposure to the open arm.

As the anxiety-related behavior has been frequently test specific, we aimed to investigate in the current study whether exposure to the open arm induces specific behavioral differences that may sustain the neurobiological differences observed in HAB and LAB rats. For this purpose, HAB and LAB rats were placed on the open arm, and a clear-cut recording of a variety of behavioral parameters was achieved. We analyzed the data using a discriminant analysis to (a) test the accuracy of divergence between the two lines and (b) determine the main factor that discriminated HAB and LAB rats on the open arm. A principal component analysis was finally performed to dissect the specific emotional dimensions in each line.

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## Method

All experimental protocols were designed to minimize rat suffering and were conducted in accordance with the European Community Council Directive (10/24/1986; 86/609/EEC).

### Rats

All rats tested were derived from two Wistar rat lines selected and bred in Munich, Germany (Landgraf & Wigger, 2002; Liebsch et al., 1998). HAB ( $n = 9$ ) and LAB ( $n = 15$ ) male rats of the 12th generation that weighed 300–400 g were kept separately in plastic cages (3–5 rats per cage) at  $22 \pm 1$  °C and 60% humidity in a 12-hr light–dark cycle (lights on at 7:00 a.m.) with food and water ad libitum. All the experiments were done between 8:30 a.m. and 12:30 p.m.

### Apparatus and Procedure

The open arm derived from the elevated-plus maze in which access to the neutral zone and the closed arms of the maze was prevented by a bar. The open arm (50 × 10 cm) was divided into a distal (25 × 10 cm) and proximal (25 × 10 cm) zone. Rats were placed in the middle of the open arm for 5 min, and their behavior was videotaped to be analyzed with an automatic videotracking system (Vidéotrack, Viewpoint, Mont d'or, France). The behavioral parameters scored were as follows: (a) number of entries and time spent (expressed as the percentage of total time) into the proximal zone of the open arm, (b) number of entries and the time spent (expressed as the percentage of total time) in the distal zone of the open arm, (c) number of rearings, (d) number of stretched attends, (e) number of head dips, (f) time spent in immobility, and (g) distance traveled.

### Statistical Analysis

Data obtained in the open arm, expressed as mean plus or minus standard error of the mean, were analyzed by a Mann–Whitney  $U$  test. In each case,  $p < .05$  was considered as statistically significant. The discriminant analysis was performed to determine the best parameter that could discriminate HAB and LAB rats. The different parameters given by this analysis were reported as follows: *classification matrix*, used to determine to which line each rat that was tested most likely belonged (results expressed as a percentage); *discriminating power of each parameter* given by the  $F$  values, indicated statistical significance in the discrimination between groups; *tolerance value*, measured the redundancy for a variable with all previous parameters included in the statistical model (a high tolerance value indicated a low redundancy between the measured parameters).

The principal component analysis was performed to complete the previous statistical analysis. The parameters chosen were as follows: (a) number of entries and time spent in the distal zone of the open arm, (b) number of rearings, (c) number of stretched attends, (d) number of head dips, (e) time spent in immobility, and (f) total distance traveled. These parameters were chosen only because they exhibited a correlation inferior to 0.9 as previously described (Salomé et al., 2002). Considering the sample size, a Spearman correlation matrix, which is less sensitive for single outliers, was used (Gorsuch, 1998). An orthogonal rotation (varimax) of the factor matrix was used to analyze the data, which ensured that the extracted factors were independent. Only factors with eigenvalues greater than 0.6 were kept for analysis.

## Results

### Open Arm Exposure

The HAB rats exhibited a differential behavior in the open arm compared with LAB rats (see Figure 1). They spent significantly less time in the distal zone ( $U = 0.0$ ,  $p < .001$ ) than LAB rats. The

number of entries in the distal zone ( $U = 23.5$ ,  $p < .05$ ) was lower in HAB rats than in LAB rats. The number of stretched attends ( $U = 24.0$ ,  $p < .001$ ) was also significantly lower in HAB rats than in LAB rats, whereas the number of head dips was lower in HAB rats ( $U = 25.5$ ,  $p < .05$ ). In addition, HAB rats tended to be more immobile than LAB rats ( $U = 37.5$ ,  $p = .07$ ). Finally, no significant line differences were observed in the distance traveled and the number of rearings.

### Discriminant Analysis

The percentage of correct discrimination as well as the discriminating factors between HAB and LAB rats are reported in Table 1 (with their corresponding  $F$  and  $p$  values and tolerance values). HAB and LAB rats were markedly divergent with a high percentage of discrimination, 100% and 93.3% in the open arm, respectively (see Table 1). The most important parameter that discriminated them in the open arm was the percentage of time spent in the distal zone.

### Principal Component Analysis

Seven parameters were selected and analyzed. The factor analysis identified three factors that distinguished HAB and LAB rats, which accounted for 85% and 77% of the total variance, respectively (see Table 2 for behavioral elements with high loadings). As the different factors were orthogonal to each other, it was generally assumed that they reflected distinct biological phenomena. To facilitate interpretation of the data, we present only the most significant loadings (higher than 0.6) in Table 2. In HAB rats, the first factor termed “anxiety” represented 52.4% of the total variance, the second factor “locomotor activity” represented 17.5% of the total variance, and the third factor “exploratory motivation” represented 15.1% of the total variance. In LAB rats, the first factor named “locomotor activity” represented 44.5% of the total variance, the second factor “anxiety” represented 18.1% of the total variance, and the third factor “risk assessment” represented 14.6% of the total variance. In HAB rats, “anxiety” was strongly related to the number and the time spent in the distal zone, the number of stretched attends, and the distance traveled; whereas in LAB rats, additionally to the percentage of time spent in the distal zone, the dimension “anxiety” had high loading for the time spent immobile. The second dimension “locomotor activity” had high loading for the number of head dips and the time spent in immobility in HAB rats, whereas in LAB rats, this factor was strongly correlated with the number of entries in the distal zone, the distance traveled, the number of rearings, and the number of head dips. In HAB rats, the third dimension “exploratory motivation” was related to rearing. In contrast, in LAB rats, stretch attends correlated with the third factor, named consequently “risk assessment.”

## Discussion

The current study provides the first precise and complete description of HAB and LAB rats' behavior following forced exposure to the open arm, currently used as a mild stressor in neurobiological studies. In all the behavioral parameters considered and analyzed, HAB rats displayed a higher anxiety-related behavior

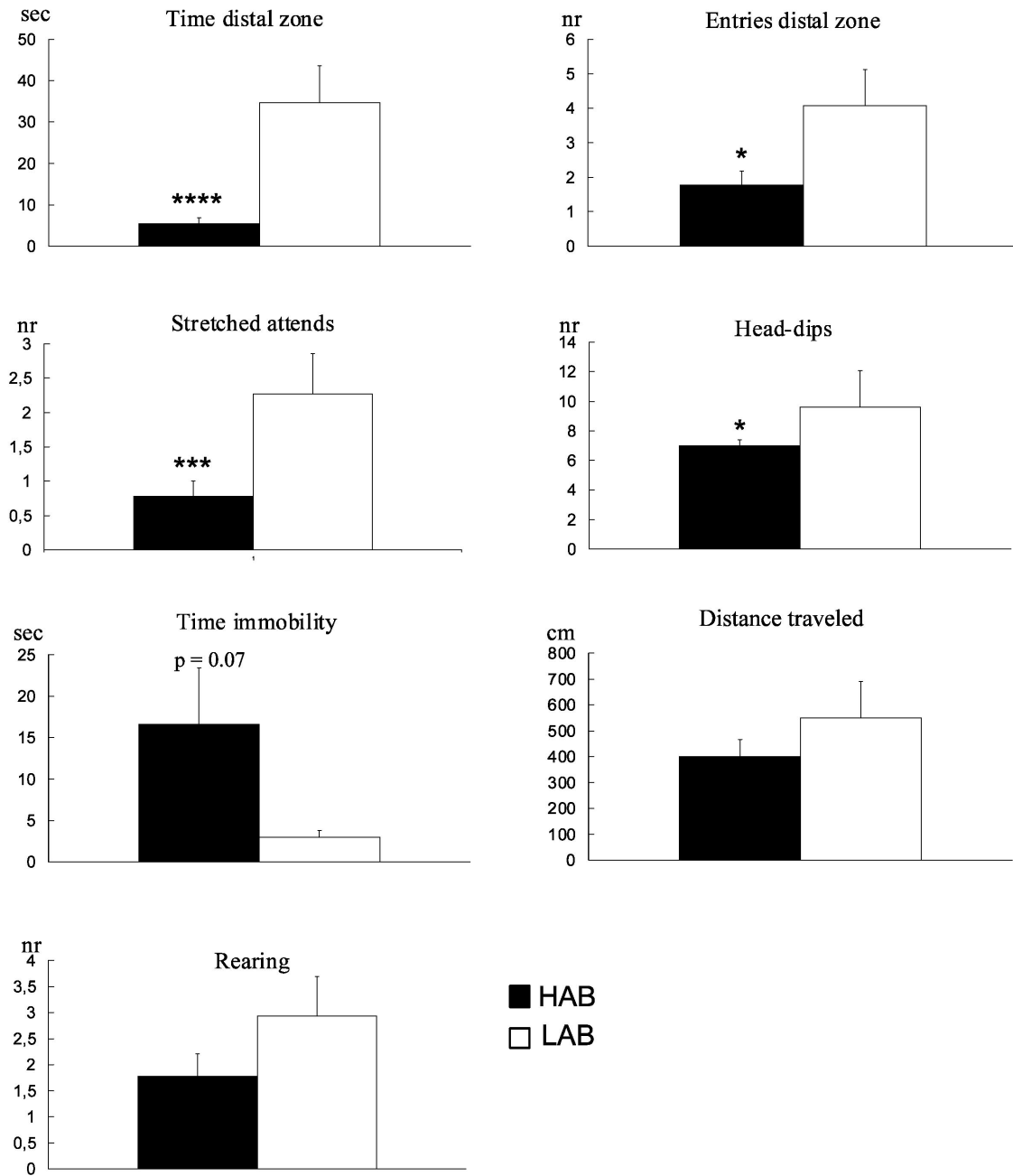


Figure 1. Behavioral parameters of high-anxiety-related behavior (HAB) rats and low-anxiety-related behavior (LAB) rats measured in the 5-min exposure to the open arm. Values are expressed as mean plus or minus the standard error of the mean for the 9 HAB rats and 15 LAB rats tested. nr = number. \* $p < .05$ . \*\*\* $p < .005$ . \*\*\*\*  $p < .001$ .

than LAB rats. Moreover, the discriminant analysis strongly confirmed that distinction between the two lines was primarily based on anxiety. Finally, the principal component analysis revealed that HAB and LAB rats presented a differential organization of their emotionality, thus validating the mild stressor as an appropriate tool to investigate and to dissect the dimensions of anxiety characterizing these two lines.

The behavior displayed by HAB rats in the open arm can be compared with data obtained in several tests of unconditioned

anxiety (Henniger et al., 2000; Landgraf & Wigger, 2002; Salomé et al., 2002; Wigger, Loerscher, Weissenbacher, Holsboer, & Landgraf, 2001). Here, HAB rats spent less time than LAB rats in the distal zone, which is considered as the unprotected area of the open arm. The distal zone can be compared with the central zone of the open field, which is thought to be potentially threatening (Liebsch et al., 1998; Salomé et al., 2004). Thus, the time spent in the distal zone of the open arm allowed us to evaluate avoidance of the unprotected area and, consequently, the thigmotaxis (which

Table 1  
*Discriminant Analysis of the Behavioral Performances of HAB and LAB Rats in the 5-min Open Arm Exposure*

Discriminating parameters	<i>F</i> (1, 16)	<i>p</i>	Tolerance	Correct %	
				HAB	LAB
% time in distal zone	7.16	.016	.72	100	93.3
Distance traveled	2.51	.13	.20		
Entries distal zone	2.18	.16	.22		
Stretched attends	1.06	.20	.85		
Time immobility	0.44	.56	.94		
Head dips	0.13	.71	.60		
Rearing	0.004	.95	.65		

*Note.* For each test, the percentage of correct classification for high-anxiety-related behavior (HAB) and low-anxiety-related behavior (LAB) is given. Each variable is organized following the discriminating power indicated by the *F* value. The corresponding *p* level is given for each *F* value. The tolerance value is a measure of the redundancy of a variable with others of the same test (a higher tolerance indicates lower redundancy).

is considered as a valuable index of anxiety). Moreover, the number of head dips and stretched attends—two parameters that have classically been related to exploratory behavior and risk assessment, respectively (Ohl, Toschi, Wigger, Henniger, & Landgraf, 2001)—were lower in HAB rats than in LAB rats. Such marked differences have been observed in studies demonstrating that among a variety of behaviors, exploratory behavior and risk assessment have been reduced in hyper-anxious rats compared with other rat lines (Griebel, Rodgers, Perrault, & Sanger, 1997; Lister, 1990). Concerning the general locomotor activity, HAB and LAB rats did not present any major difference, as the distance traveled was similar during the 5-min exposure to the open arm. However, this result is in discrepancy with those previously reported in the open field, in the modified hole board, and in the black and white box tests (Henniger et al., 2000; Ohl et al., 2001; Salomé et al., 2004) in which the locomotor activity was higher in LAB rats than in HAB rats. This may be due to, at least partially, the size of the open arm in this study compared with the other cited tests. In fact, in these tests, rats easily moved around in the large arena. Thus, differential locomotor activity between HAB and

LAB rats could be more precisely dissected. Finally, even if HAB and LAB rats traveled the same distance in the open arm, the time spent in immobility tended to be higher in HAB rats than in LAB rats, indicating that HAB rats were less active than LAB rats. This difference might be interpreted in part in terms of anxiety-related behavior, as movement inhibition may be considered as one characteristic of anxious rats (Escorihuela et al., 1999; Henderson, Turri, DeFries, & Flint, 2004; Rodgers, Cao, Dalvi, & Holmes, 1997). Overall, our data sustained that forced exposure to this mild stressor generated a high level of anxiety in HAB rats, which was also revealed by neuroendocrine data (Landgraf et al., 1999).

The discriminant analysis reinforced these observations. The highest probability to differentiate HAB rats from LAB rats was resumed to only one parameter closely related to trait anxiety, that is, the percentage of time spent in the distal zone of the open arm, whereas the locomotor activity was not relevant in discriminating these two lines. The principal component analysis used in this study permitted us to identify three emotional dimensions in both HAB and LAB rats. The two first dimensions represent differential percentages of the total variance between the two lines, indicating a clear differentiation in emotional dimensions between both lines. In HAB rats, the most important dimension was anxiety (which explained 52.4% of the total variance), whereas in LAB rats, the most important dimension was locomotor activity (which explained 44.5% of the total variance). Thus, HAB rats and LAB rats seem to differentially evaluate the forced exposure to the open arm. Such appraisal could lead to the differential Fos expression observed in our previous study (Salomé et al., 2004). Finally, the third and last dimension was not similar in the two lines. In HAB rats, the dimension extracted was named “exploratory motivation” (rearing) and in LAB rats “risk assessment” (stretch attends), indicating again that these two lines presented differential appraisals of a new and mild stressful environment. Overall, forced exposure to the open arm revealed a higher level of anxiety-related behavior displayed by HAB rats compared with LAB rats and induced a stress-coping strategy that strongly differed between HAB and LAB rats, with the former exhibiting a more passive behavior. Such passive behavior was also observed in nonsocial and social stress tests, including the forced swimming test, the modified hole board test, and the social defeat test (Frank et al.,

Table 2  
*Principal Component Factor Analysis Performed With the Open Arm Exposure Behavioral Data*

Emotional dimension	HAB			LAB		
	Behavioral parameters	Factor	% of variance	Behavioral parameters	Factor	% of variance
Anxiety	% time DZ (+) Entries DZ (+) Stretch attends (–) Distance traveled (+)	1	52.4	% time DZ (+) Time immobility (–)	2	18.1
Locomotor activity	Head dips (+) Time immobility (+)	2	17.5	Entries DZ (+) Distance traveled (+) Rearing (+) Head dips (+)	1	44.5
Exploratory motivation Risk assessment	Rearing (+)	3	15.1	Stretched attends (+)	3	14.4

*Note.* Only behavioral parameters with loadings greater than 0.6 are shown. Plus and minus signs indicate the direction of the particular loading. HAB = high-anxiety-related behavior; LAB = low-anxiety-related behavior; DZ = distal zone of the open arm.

2006; Landgraf & Wigger, 2002), suggesting that HAB rats' coping style is not stressor quality dependent (strength and duration) but rather is more related to their trait (i.e., innate) anxiety. This study further confirmed that the emotional parameter that has strongly discriminated HAB and LAB rats was associated to anxiety, reinforcing the notion of their robust innate anxiety. As the behavioral consequences of exposure to the open arm have now been fully validated for HAB and LAB rats, exposure to the open arm should be regarded as a useful tool to dissect more precisely the neurobiological substrates underlying the extremes in anxiety-related behavior.

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