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Research report

Individual response profiles of male Wistar rats in animal models for anxiety and depression

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Abstract

Previous work has shown that systematic individual differences between male Wistar rats can be detected in tasks like the elevated plus-maze, or the open field. Here, we investigated whether individual profiles of anxiety, as measured with the plus-maze, may predict behavioral response profiles in other tasks where anxiety, aversion, or depressive behaviors are important. Male Wistar rats were initially screened: (A) in an open field; and (B) in an elevated plus-maze. Based on their plus-maze behavior, that is, the time spent in the open arms, the animals were divided into two subgroups with either 'low' or 'high' anxiety (LA or HA) levels. These subgroups were exposed to other experimental anxiety paradigms, namely object burying and two-way active avoidance, and an animal model of depression, the forced swim test. In the plus-maze, the percentage of time spent on, and the number of entries into the open arms were lower in HA than in LA rats. In the object burying task, HA rats showed more burying behavior of Tabasco-coated marbles, and in the active avoidance task, they showed slower acquisition of avoidance learning and higher escape latency as compared to LA rats. Finally, LA and HA rats behaved similarly in the forced swim test; however, the percentage changes of immobility time between test days 1 and 2 were negatively correlated to open field behavior, namely locomotor activity and center entries. On the other hand, the frequencies of rearing in the open field, which can also gauge functional differences between rats (for example responsiveness to novelty, psychomotor activation), were not substantially related to the behavioral profiles in the tests of anxiety and depression. These results show that individual differences of anxiety in the plus-maze can be predictive of behavior in other anxiety models, but not in forced swim test, indicating that they may be determined partly by similar functional and physiological mechanisms.

Keywords: Individual differences; Elevated plus-maze; Burying behavior; Active avoidance; Forced swim; Anxiety; Open field; Serotonin

1. Introduction

In the field of experimental anxiety research, the elevated plus-maze is a widely used behavioral paradigm [49], which presumably measures fear-motivated avoidance behavior [24,40]. During a typical plus-maze test, animals will spend most of the time in the closed arms of the maze; a behavior, which seems to reflect their fear of open spaces [58]. Anxiolytic drug treatments are known to decrease this natural aversion toward the open arms, whereas anxiogenic drugs can enhance it [40]. Apart from these general patterns, however, several experiments have shown that rats, although identical in strain, sex, and age can differ systematically in their behavioral response to an elevated plus-maze [8,51]. In addition, pharmacological experiments also show that this paradigm yields a wide range of, often contradictory, results [28].

Variability in the plus-maze may at least partly be due to inherent differences between rats within a given strain. Thus, we have recently shown that, based on the time spent in the open arms of the maze, male Wistar rats can be divided into two subgroups with low (LA) or high anxiety (HA) levels. These LA and HA rats differed in serotonin (5-HT) tissue levels in the ventral striatum, but not in the neostriatum, frontal cortex, amygdala or ventral hippocampus. Furthermore, LA and HA rats did not differ with respect to norepinphrine or dopamine

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(DA) in these brain areas [51]. Interestingly, the ventral striatum, which includes the nucleus accumbens, is known to play a critical role for motivated behavior, and 5-HT is a transmitter, which seems to mediate anxiety [23]. Furthermore, we found that anxiety in the plus-maze was not related to rearing or locomotor behavior in the open field test, that is, measures which can also detect systematic differences between rats [55,56]. These behaviors, however, seem to reflect largely DAergic and cholinergic brain mechanisms in relation to psychomotor activation, novelty responding, motivation, addiction and stress [7,35,42].

Here, we asked whether individual differences of anxiety in the elevated plus-maze are related to responses in other tests, where behavior is also determined by anxiety or aversion. Thus, we screened rats in the plus-maze and open field, and then tested them in specific other tasks, namely object burying, two-way active avoidance and forced swimming. If individual differences in the plus-maze can predict anxiety-like responses in such other animal models, plus-maze screening could serve as a valuable research tool, since it would help to differentiate between animals with specific expressions of functional and/or physiological mechanisms.

Object burying is a rodent-specific defence reaction to aversive stimulation [43,59], and it is an active strategy to cope with a stressor, which can be measured in a home cage situation. In this paradigm, rats push bedding material toward or over the aversive stimuli. This behavior is suppressed by anxiolytic drugs [57,59] and is therefore taken as an indicator of anxiety [6,58]. Active avoidance is another model of anxiety [15]. When animals face an avoidable stressful event, most of them try to actively cope with it by performing an appropriate response, leading to escape from the aversive stimulus, whereas a minority of animals tend to freeze more in such a situation [14]. In the two-way active avoidance paradigm used here, animals can escape from the aversive stimulus by crossing to the opposite side of the testing apparatus. With repeated trials, most rats learn to avoid the aversive stimulus by crossing to the other side contingent on an otherwise neutral stimulus, which precedes the occurrence of the aversive stimulus. Although anxiolytic manipulations which decrease serotonergic function can improve active avoidance performance [13,41], there are individual differences in the sensitivity of avoidance behavior to anxiolytic agents [3,32,54].

Since its introduction by Porsolt et al. [44] the forced swim test has been widely used to screen the clinical efficacy of antidepressant drugs. However, the limitation of this animal model has been discussed since selective 5-HT reuptake inhibitors, which are an important class of antidepressant drugs in clinical practice, are rather inactive in this test [4]. Compared with anxiety tasks, forced swimming is an inescapable stress. When animals are forced to swim in a restricted space, they initially display escape-oriented struggling [1,37]; over time, they gradually adopt an immobile posture. Because the internal anxiety level may have certain correlation with the ability to cope with the environmental challenge, we also analyzed the role of individual anxiety levels in behavioral strategies adapted to either inescapable or escapable stressful situations.

The present experiment was designed to obtain more detailed behavioral information on rats with individual differences in anxiety in the plus-maze, or rearing in the open field. For this purpose, rats were initially tested in a similar way as in our previous studies [51,56], that is, they were first screened in an open field, and then in a plus-maze. Based on plus-maze behavior, they were divided into LA and HA groups. The major objective was to analyze possible relationships between plus-maze anxiety and reactivity in other models of anxiety and depression. The second aim was to examine the relationship between anxiety level and coping behavior in escapable (burying and two-way active avoidance tests) and inescapable (forced swim test) situations.

2. Materials and methods

2.1. Animals

All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals. Forty male Wistar rats (Harlan Winkelmann, Borchen, Germany) were used and housed in groups of five rats in acrylic cages ($35 \times 56 \times 19$ cm) in an animal room with a 12 h light–dark cycle (lights on at 07:00 h) with food and water provided ad libitum. The mean body weight was 325 ± 2 (SEM) and 397 ± 4 g at the beginning and the end of this study, respectively. Each animal was handled for 5 min on 6 consecutive days prior to the experiment.

2.2. General procedure

Three tests were performed in the following order: open field, elevated plus-maze, and object burying. Furthermore, the rats were tested for two-way active avoidance and in a forced swim test. Here, we used a counterbalanced order of testing, since it has been shown that acute exposure to an unavoidable stressor may temporarily impair subsequent avoidance patterns [36,50]. Therefore, one might expect that the experience of forced swimming might critically affect subsequent active avoidance behavior, or vice versa. In order to account for these possible interactions, we split our sample of animals into two equal-sized subgroups (irrespective of individual plus-maze or open-field behavior). One subgroup received the active avoidance test first, followed then by the forced swim test, whereas the other group was tested in the reversed order. The intervals between these behavioral test paradigms were 7 days. All behavioral tests were started 3 h after the start of the lights-on period (10:00 h). First, the animals were weighed in the animal room. Then, they were placed individually in a clean cage ($25 \times 41 \times 19$ cm) and transported to a dim observation room. Defecations during behavioral testing were scored. The pieces of test equipment were thoroughly cleaned by using water and 20% alcohol followed by thorough drying before each rat was tested. The behavioral parameters were analyzed by an automated computer program or by scoring from videotapes.

2.3. *Behavioral tests*

2.3.1. *Open field*

The open field consists of an acrylic box ($40 \times 40 \times 40$ cm) which was monitored by an automated activity monitoring system (Tru Scan^{TM}, Photobeam Sensor-E63-22; Coulbourn Instruments; USA). One grid of infrared sensor beams was mounted horizontally 3 cm above the floor, and a second tier of beams was mounted 16.5 cm above the floor to measure vertical (rearing) activity. Additionally, a video camera was suspended 150 cm above the center. Open field activity was measured under conditions of red light (28 lux).

The following measures were obtained using the Tru Scan system: (1) rearing number; (2) locomotion: the distance traveled in cm; (3) center time: defined as the animal's center of gravity being within the center area of the open field (20×20 cm); (4) center entry: the number of entries into the center area of open field. Behavior in the open field was tested twice on 2 consecutive days (10 min each).

2.3.2. *Elevated plus-maze*

The elevated plus-maze apparatus was made of plastic and consisted of two opposed open arms (50×10 cm), two opposed enclosed arms with no roof ($50 \times 10 \times 40$ cm), and an open square (10×10 cm) in the center. The maze was elevated 50 cm above the floor. The behavioral test was conducted in the observation room using the same level of illumination as used for the open field test. The animals were placed into the center of the plus-maze, facing one of the open arms. The following measures were analyzed from videotapes: (a) the number of entries into and (b) the time spent on open or enclosed arms; (c) rearing; (d) grooming; (e) risk assessment; and (f) open arm latency, that is, the time from placing the rat into the plus-maze until it entered one of the open arms. An entry into any of the compartments was defined as all four paws being placed on the arm. Each rat was tested on 2 consecutive days (5 min each).

2.3.3. *Object burying*

The test of burying behavior was performed in the animal housing room. Each rat was placed singly into an acrylic cage ($25 \times 41 \times 19$ cm) with a bedding of 5 cm of fine sawdust (Altromin WH 3-4). There, it was kept for a 1-day habituation period. Then, four glass marbles (3.5 cm in diameter) were placed in a row next to one of the 25 cm walls of the cage. In addition to a previous procedure of object burying [43,59], each marble was covered with a drop of Tabasco pepper sauce (Mc. Ilhenny Co., USA) to increase its aversiveness. The status of the marbles was monitored and videotaped over a period of 22 h to determine the topography and time course of burying. During the first hour, measures were taken every 15 min; thereafter, they were taken every hour. At 19:00 h, the main lights of the animal room were turned off, and red light (127 lux) was turned on. In the subsequent first hour of the dark phase, burying behavior was again monitored every 15 min. The following parameters were recorded: the number of rats showing burying behavior, and the number of buried marbles (surface covered with sawdust by more than 50%).

2.3.4. *Two-way active avoidance*

The active avoidance test was carried out in the observation room under 900 lux illumination, using a two-way shuttle-box (33×66 cm wide, 39 cm high). The floor was made of 2 mm diameter stainless steel rods spaced 1.5 cm apart. The box was divided into two equal compartments by a 5 cm high Plexiglas barrier. Each compartment could be electrified separately through a shock scrambler (521/C, Camden Instruments). A speaker was mounted in the center on the top of the box for delivery of auditory stimuli. The animals were placed into the shuttle-box and allowed to explore the entire apparatus for 2 min. Then, they received 20 shuttle trials, where they were allowed to terminate a shock by jumping over a barrier to the adjoining compartment. Each trial began with a 115 db tone, which lasted 3 s and which was followed by a 0.3 mA scrambled foot shock. If the animal crossed the barrier during the tone, the stimulus was terminated and no shock was delivered (avoidance response). If the animal crossed the barrier during shock delivery, an escape response was measured. If the rat failed to cross, the shock was terminated after 15 s (escape failure). After 42–60 s, the next trail was initiated. The latency to avoid or escape, and the number of avoidances, escapes and failures were recorded.

2.3.5. Forced swim

This test was carried out in a clear glass tank (25 × 25 × 60 cm) containing 39 cm clean water (26 °C). The apparatus was cleaned thoroughly, and water was changed from rat to rat. Two swimming sessions were conducted and videotaped: 15 min on the 1st day and 5 min on the 2nd day. After each test, the rats were dried and kept warm under a heating bulb for 30 min before being returned to their home cages. The following parameters were measured from videotapes: (1) struggling, that is, strong movements of the limbs occurring during diving, breaking the surface of the water, or scratching the walls, and (2) immobility, which occurred when the rats remained motionless, or floating (including small limb movements to keep their heads above the water [1].

2.4. Data analysis

Identically to our previous experiments [51,56], the animals were ranked using the relative time spent in the open arms (expressed as percentage of total observation time) and were assigned to two subgroups with either high (the 19 animals with the lower percentage in the open arms; HA rats) or LA levels (the other 19 animals with the higher percentages; LA rats). Two animals were excluded from further analysis since they dropped from an open arm of the plus-maze. These group assignments were used to present all other behavioral data.

All results were expressed as the mean ± SEM. Statistical testing was performed to compare within or between groups using t-tests for paired or unpaired data. Furthermore, a two-way analysis of variance (ANOVAs) with repeated measures followed by Scheffe's test was applied to analyze the results of active avoidance and object burying test, which involved measures across a number of repeated observations. To test for correlations between behavioral variables, Pearson's correlation coefficient was applied. The level of significance was defined as $P < 0.05$.

3. Results

Based on the measure of time spent in the open arms of the plus-maze (day 1; expressed as percentage of total observation time), animals were assigned to the HA and LA subgroups. These subgroups had the following profiles:

3.1. Elevated plus-maze test

The percentage of open arm time, which was used to differentiate the animals on day 1, was still higher in LA than HA rats on the 2nd day of testing ($t = 2.6$, df = 36, $P = 0.013$). In both groups, the percentage of open arm time declined from the 1st to the 2nd day (HA rats: $t = 2.7$, df = 18, $P = 0.02$; LA rats: $t = 7.0$, df = 18, $P < 0.001$). Besides open arm time, the number of open arm entries was also higher in LA rats (day 1: $P < 0.001$, day 2: $P = 0.02$), whereas the number of entries into the closed arms did not differ substantially between groups. The latencies until the first entry to an open arm also differed between HA and LA rats, with shorter latencies in LA rats (day 1: $t = 3.0$, df = 35, $P = 0.005$; day 2: $t = 2.1$, df = 36, $P = 0.047$).

LA rats showed less rearings in closed arms than HA rats (day 1: $t = 2.3$, df = 36, $P = 0.03$), together with a trend for more rearing in the open arms ($t = 1.9$, df = 36, $P = 0.07$). Furthermore, LA rats showed less risk assessment than HA rats on the 1st day ($t = 3.1$, df = 36, $P = 0.004$), but not on the 2nd day. However, when expressing risk assessment as a percentage of closed arm time, there were no longer differences in risk assessment between HA and LA rats (day 1: HA rats 12.6 ± 1.5%, LA rats 10.1 ± 1.2%). Finally, the analysis of grooming behavior did not yield indications for differences between groups, except for an increase in grooming behavior from days 1 to 2 in LA rats ($P = 0.018$; Table 1).

3.2. Open field test

In contrast to the plus-maze, none of the measures obtained in the open field (Table 2) yielded significant group differences between LA and HA rats within one testing day (unpaired t-tests; all P-values > 0.05). However, when comparing behavior on days 1 and 2, between-session habituation of rearing, locomotion, and center time was observed in LA (paired t-test, all P-values < 0.05) but not in HA rats.

3.3. Object burying test

Within a few minutes after marble placement, almost all rats explored, touched, or sniffed the marbles with their snout, and rapidly withdrew it thereafter. Some animals then started to spray and push a pile of bedding material toward the marbles with their forepaws and snout; however, most of the animals showed burying behavior later on. About 30% (six out of 19) of the LA rats and 50% of the HA rats showed burying behavior within the first 2 h; however, there were no statistical differences between these percentages. Initially, after the lights had been shut off, the percentage of animals showing burying behavior were still similar between HA and LA rats, and increased gradually to more than 90% in both groups at the end of this testing period (Fig. 1A). In contrast, the average number of marbles buried was higher in HA than LA rats during the 1st 2 h after introducing the marbles, ($F_{1,36} = 4.11$, $P < 0.05$; Fig. 1B).

Table 1
Plus-maze behavior

Group	Open arms							
	% Open arm time		Latency (s)		Entries (no.)		Rearing (no.)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
HA rats	24.7 ± 2.0	15.7[a] ± 3.4	21.8 ± 4.8	39.8 ± 16.7	4.7 ± 0.4	4.1 ± 0.7	0.6 ± 0.2	0.2[a] ± 0.1
LA rats	48.9*** ± 2.4	27.5*[c] ± 3.0	6.4** ± 2.3	5.3* ± 1.4	8.0*** ± 0.4	6.2*[c] ± 0.5	1.3 ± 0.3	0.3[a] ± 0.2
	Closed arms							
	Entries (no.)		Rearing (no.)		Risk assessment (s)		Grooming (s)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
HA rats	8.9 ± 0.7	9.1 ± 0.4	18.3 ± 1.0	17.8 ± 1.6	23.4 ± 2.9	24.2 ± 3.2	8.0 ± 2.5	11.9 ± 3.6
LA rats	8.1 ± 0.6	7.7 ± 0.8	14.8* ± 1.1	14.6 ± 1.3	12.5** ± 2.0	24.4[b] ± 3.8	7.1 ± 3.0	12.6[a] ± 2.7

Data reflect mean ± SEM obtained in two 5-min test sessions that were performed on 2 consecutive days. *, **, and *** $P < 0.05$, 0.01, and 0.001, between group comparison with HA rats, t-test; [a], [b], and [c] $P < 0.05$, 0.01, and 0.001, between session comparison with day 1, paired t-test.

3.4. Counterbalance of active avoidance test and forced swim test

Apart from the number of boli counted during the active avoidance test (6.0 ± 0.7 with rats which had experienced the forced swim earlier vs. 8.1 ± 0.5 in animals which had not; $P = 0.023$), there were no behavioral differences in active avoidance behavior or forced swimming between animals which had been tested for active avoidance before the forced swim test vs. those which had been tested in the reversed order (P-values > 0.05). Therefore, the respective data from these two subgroups were pooled together and will be presented in the pooled manner in the following.

3.5. Two-way active avoidance

One rat from the LA group was excluded from further analysis because this rat managed to jump and remain on the barrier separating the two halves of the testing apparatus, instead of shuttling between them.

The escape latencies of the remaining LA and HA rats decreased gradually across trials ($F = 18.41$, $P < 0.001$) and reached an asymptotic level of about 3 s after 10 trials, indicating that the animals learned to escape from foot shock (Fig. 2). Further indications for learning were provided by the measure of avoidances (Table 3), since the number of successful avoidances upon exposure of the conditioned stimulus was higher during trials 11–20 than during trials 1–10 (HA rats: $t = 7.4$, df = 18, $P < 0.001$; LA rats; $t = 6.0$, df = 17, $P < 0.001$).

Indications for significant differences between HA and LA rats were obtained in the measure of avoidance number which was higher in LA than in HA rats during the first 10-trials ($t = 3.2$, df = 35, $P = 0.003$). The escape latencies (Fig. 2) did not differ significantly between LA and HA rats ($F = 0.40$, $P = 0.531$); however, there was an initial trend for shorter latencies in LA rats, especially during the second trial. The numbers of escapes, failures, and fecal boli in this test did not differ between groups (Table 3).

3.6. Forced swim test

The time of immobility during the first 5-min period increased from days 1 to 2 (LA rats: $t = 4.2$, df = 18, $P = 0.001$; HA rats: $t = 2.1$, df = 18, $P = 0.026$). There were no differences between HA and LA rats in this measure, neither on day 1 nor on 2 (Fig. 3).

Table 2
Open field behavior

Group	Rearing (no.)		Locomotion (m)		Center time (s)		Center entries (no.)		Boli (no.)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
HA rats	47.9 ± 3.4	42.4 ± 3.7	24.38 ± 1.23	23.29 ± 1.05	333.6 ± 21.5	298.3 ± 24.7	73.2 ± 4.9	72.8 ± 3.6	4.4 ± 0.5	3.4[a] ± 0.5
LA rats	49.7 ± 3.6	40.2[b] ± 1.9	26.61 ± 1.08	23.43[b] ± 0.83	381.4 ± 21.5	328.0[a] ± 17.8	78.7 ± 3.9	77.0 ± 3.7	5.1 ± 0.6	4.3 ± 0.6

Data reflect mean ± SEM obtained in two 10-min test sessions that were performed on 2 consecutive days. [a], and [b] $P < 0.05$, and 0.01, between session comparison with day 1, paired t-test.

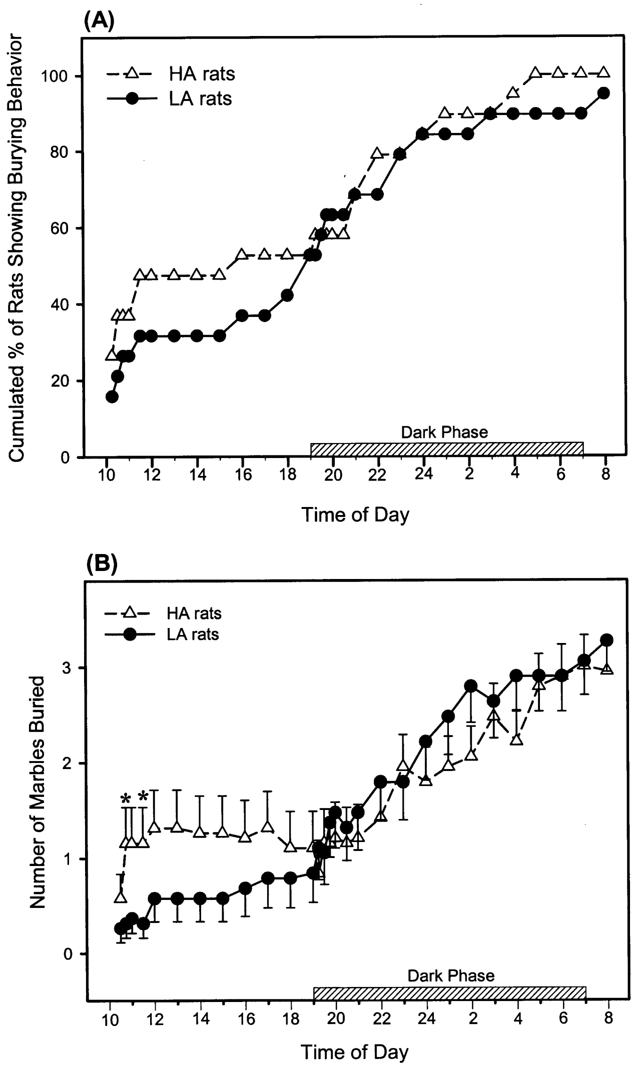

Fig. 1. Percentage of rats showing burying behavior (A) and the number (mean ± SEM) of marbles buried (B) displayed over time. Four marbles coated with Tabasco sauce were introduced into rat's cage at 10:00 h. *, $P < 0.05$, compared with LA rats at the given time point.

When calculating the percentage changes of immobility time between the 2 observation days, a negative correlation was found with measures of open-field behavior, namely locomotion ($r = -0.32$, $P = 0.042$) and center entries ($r = -0.33$, $P = 0.038$) (Fig. 4), but not with measures of plus-maze behavior. No between-group differences were noted in the time spent struggling and in the number of fecal boli (data not shown).

3.7. Analysis of animals with low (LR) vs. high rearing (HR) levels in the open field

In addition to the analysis based on plus-maze behavior, all animals were divided into HR and LR rats based on the number of rearing in the open field tested on the 1st day. When analyzing behavior in plus-maze, object burying, active avoidance and forced swim test, there were no indications for substantial differences between HR and LR rats (data not shown in detail).

4. Discussion

In the present experiment, we asked whether the behavioral profiles of male Wistar rats in tests of object burying, forced swimming, and two-way active avoidance learning are related to individual differences in the elevated plus-maze and/or the open field. The results from plus-maze and open-field testing are consistent with our previous reports [51,56]: based on the percentage of time spent on the open arms of the plus-maze, animals were differentiated into HA ('high anxiety') and LA ('low anxiety') rats. In addition to less open arm time, HA rats also entered these open arms less often. These differences in plus-maze behavior were not paralleled by differences in open-field behavior, since HA and LA rats had comparable levels of rearing and locomotor activity, which support our previous conclusions that individual differences in plus-maze and open-field behavior in male Wistar rats reflect different functional and neurochemical mechanisms [51,55,56]. In extension of our previous work, we found here that HA and LA rats differed in object burying behavior and active avoidance learning. Again, such differences were not seen in group assignments which were based on rearing or locomotor activity in the open field. In contrast, individual differences were found in the forced swim test which were related to open field, but not to plus-maze behavior, since changes in immobility in the swim test were negatively correlated to locomotor activity and center entries. Together, these results support the hypothesis that individual differences in plus-maze behavior can predict behavioral outcome in other models for anxiety but not in a model for depression.

The validity of rodent anxiety models is largely based on the effectiveness of anxiolytic agents that can affect behavior in these models in a rather specific way; however, the detailed behavioral results have been equivocal with plus-maze [5], active avoidance [58], and burying behavior [17,38]. This may be due to subject-dependent or independent factors in a given experiment, for example in animal strain, age, handling or testing procedures. However, inter-individual differences in physiological activity and anxiety levels may also play a role. Two interpretations for the present results are suggested: one is the potential involvement of behavioral strategies used by the animals to cope with stressors in a behavioral task. The other deals with the possible relationships between neurochemical and functional differences in LA and HA rats.

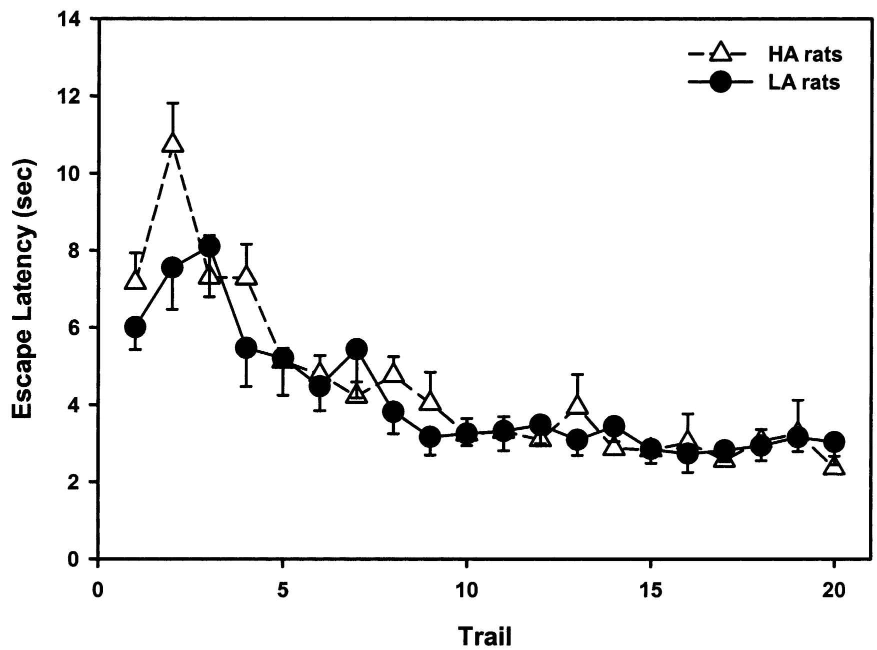

Fig. 2. Escape latencies of rats in the two-way active avoidance test. Results are expressed as the mean ± SEM latency (s) to escape from foot shock.

4.1. Elevated plus-maze test

Open arm activity in the elevated plus-maze is taken as an index of anxiety, with low levels of open arm time indicating higher levels of anxiety, and vice versa [30,40]; therefore, the different plus-maze profiles of HA and LA rats which were similar to those of our previous report [51] should reflect different levels of anxiety.

Although LA rats also showed less rearing and risk assessment in closed arms than HA rats, these two parameters may not serve as a useful index of anxiety because they depend on the time spent in the closed arms. LA rats spent less time on closed arms compared to HA rats; thus, shorter closed arm time equates to less opportunity to display behaviors on it. Actually, when expressing risk assessment as a percentage of time on closed arms, there was no longer a difference in risk assessment between LA and HA rats. In addition, the behavioral differences between LA and HA rats in the plus-maze may not be due to the general differences in locomotor activity, since there were no between-group differences in locomotion in the open field, which is consistent with our previous data [46,47]. In this respect, our HA and LA are unlike rat lines bred for differences in avoidance behavior (see also below), since these differ not only in plus-maze behavior, but also in open field locomotor activity and rearings [14]. Also, other rat lines bred for differences in plus-maze behavior were found to differ in locomotor activity [27].

4.2. Object burying test

Laboratory rats can show defensive burying to unfamiliar and/or noxious objects placed into their home cages; and this behavior also serves as a model of fear and anxiety [59]. Pinel and Treit [43] suggested that marbles could provide an effective unconditioned stimulus that provokes burying which, in turn, may indicate anxiety. The present results show that HA rats were more reactive to the marbles than LA rats, and it is suggested that HA rats may have a lower defensive threshold to such aversive or unfamiliar stimuli. In addition, although almost all rats showed burying behavior, most of them started to bury marbles during

Table 3
Behavior of rats in the two-way active avoidance test

Group	Avoidance no.		Escape no.	Fail no.	Boli no.
	1–10 trial	11–20 trial			
HA rats	1.4 ± 0.2	5.7 ± 0.5[a]	12.5 ± 0.5	0.5 ± 0.2	7.3 ± 0.4
LA rats	2.8 ± 0.4**	5.6 ± 0.5[a]	11.1 ± 0.7	0.5 ± 0.3	6.8 ± 0.7

For avoidance no., the data are expressed as the average of 10-trial blocks ± SEM. For the remaining parameters, data are expressed as the average of the total 20 trials (± SEM). ** $P < 0.01$, between group comparison with HA rats, t-test. [a] $P < 0.001$, compared with trials 1–10, paired t-test.

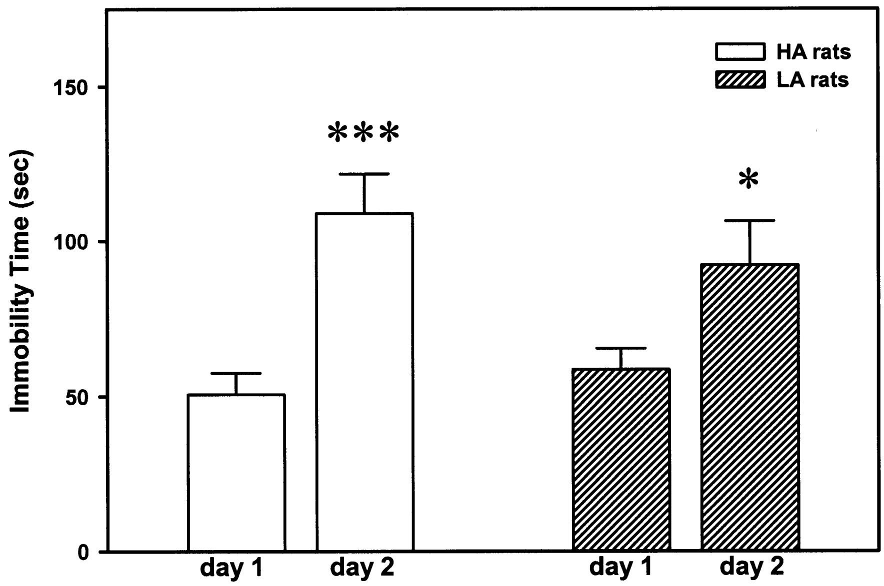

Fig. 3. Immobility time of rats in the first 5 min of the forced swim test. Data are expressed as mean ± SEM time spent on immobility (s). *, and ***, $P < 0.05$, and $P < 0.001$, compared to day 1.

the dark phase. However, topography and time course of burying were different between LA and HA rats during the light phase when HA rats buried marbles more vigorously than LA rats.

4.3. Two-way active avoidance

Avoidance acquisition in an active avoidance task has been proposed as a valid model of anxiety [16,18,20]; however, some studies testing the effectiveness of anxiolytic agents yielded inconsistent results [15,58]. The current study showed that LA rats acquired avoidance more efficiently than HA rats; HA rats initially showed a poorer performance that improved throughout consecutive sessions. This pattern of results has meanwhile been replicated by us in a separate study (Ho et al., unpublished data), which provides an indication for the robustness of our present findings. It is unlikely that these results are due to individual differences in pain reactivity since we recently also found that HA and LA rats do not differ in the tail-flinch and the hot-plate test (Borta and Schwarting, in preparation). An alternative explanation for the present findings could be that HA rats are more emotional in response to shock than LA rats, which might impair acquisition of avoidance learning in the shuttle-box [16]. Following this explanation one would argue that plus-maze behavior does not necessarily reflect a 'state'-type variable (like anxiety), but rather a 'trait'-type since it can be predictive of behavioral performance in other tests, like the active avoidance used here.

Individual differences in such mechanisms could at least partly explain why the sensitivity of active avoidance behavior to anxiolytic agents may vary between subjects. Thus, it has been shown that anxiolytic drugs can improve avoidance behavior of 'poor performers' (i.e. subjects receiving high rates of shock) and disrupt the behavior of 'good performers' (subjects receiving low rates of shock) [3,32,54].

4.4. Forced swim test

In the forced swim test, there were no between-group differences between HA and LA rats in the measure of immobility time which is usually taken as the critical measure [1,37]. This finding is in contrast to those of others [33] using Wistar rat lines bred for HA or LA. Nevertheless, we found indications for systematic differences between animals, since locomotor activity and center entries in the open field were negatively correlated with immobility in the forced swim test. Thus, factors other than those tested in the plus-maze can affect performance in the forced swim test. Although the average time of immobility in the swim test was enhanced from days 1 to 2 in both, LA and HA rats, this pattern of change between tests was not observed in all animals, since some also showed decreased immobility on day 2. When calculating these changes in terms of percentage changes, we found a negative correlation to behaviors in the open field (locomotion, center entries). These results are consistent with previous findings that low responders, according to their level of locomotor reactivity to novelty, showed increased immobility in the forced swim test [50]. Locomotion in an open field is considered to reflect general or exploratory activity, and center entries are suggested to be an indicator of emotionality in rats [21,45]; thus, changes of immobility

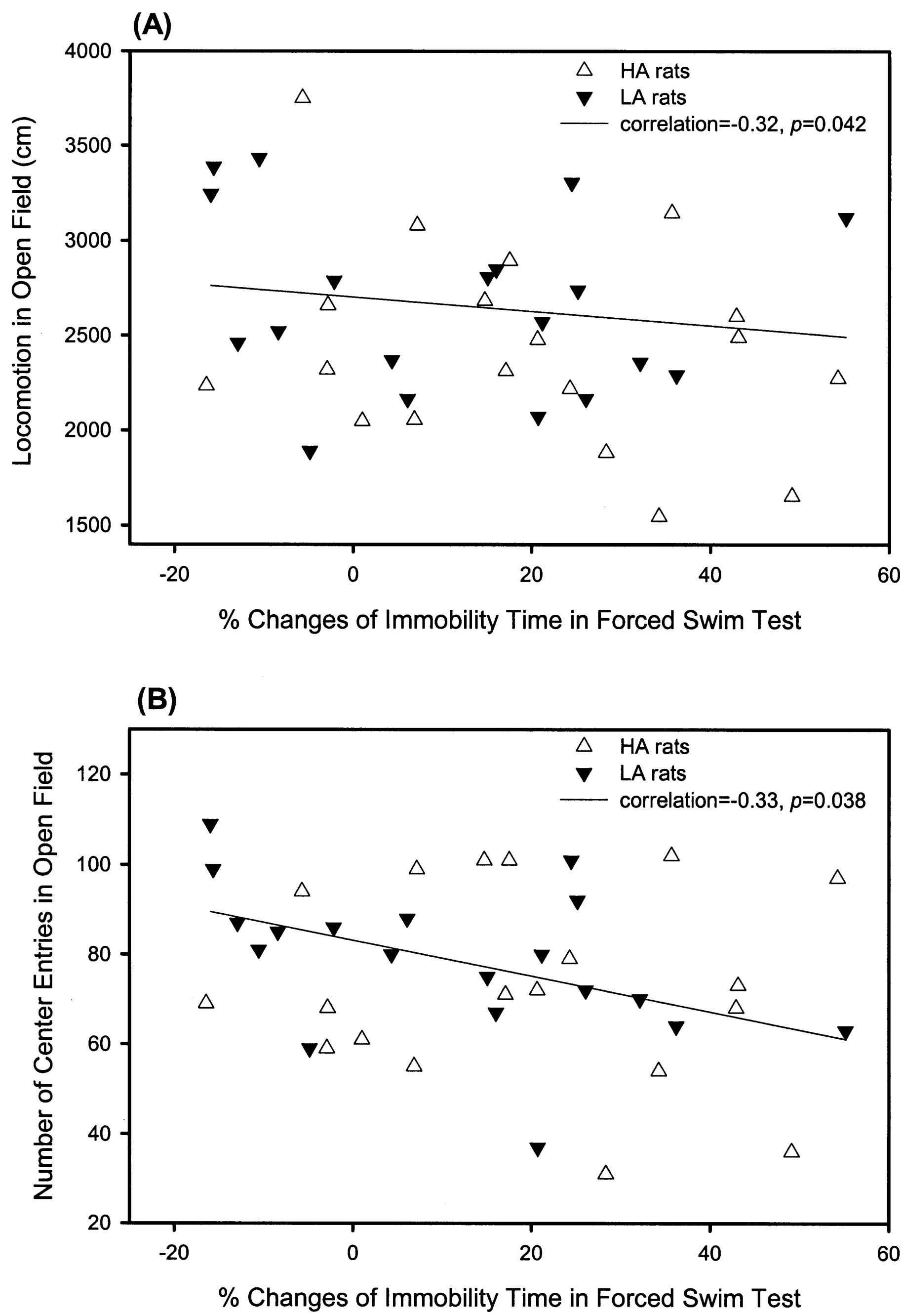

Fig. 4. Scatter plots depicting the correlations between the % changes of immobility time in the forced swim test vs. locomotion (A), or the number of center entries (B) in the open field test. The % changes of immobility time are defined as the differences of immobility time (%) during the first 5 min on days 1 and 2.

in the forced swim-test may be related to factors of exploratory activity and emotionality.

4.5. Coping strategy

When animals face a stressful event, they first try to cope with it by performing appropriate responses, for example, by escaping from the aversive stimulus. Furthermore, they display active coping strategies to escapable stressors, but passive (or inhibitory) coping to inescapable stressors. In the present elevated plus-maze, burying, and active avoidance paradigms, animals can escape from or avoid aversive or nociceptive stimuli by avoiding the open arms, by burying the marbles, or by crossing to the opposite side of the shuttle-box. In the inescapable forced swim test, animals initially display active struggling and try to escape [1,37]; over time, they gradually adopt a passive immobility. In the current study, LA rats showed less open arm avoidance in elevated plus-maze, less burying behavior to marbles, and quicker avoidance acquisition in shuttle-box, compared with HA rats. However, the LA and HA rats displayed the same levels of immobility in the forced swim test. These results indicate that the performance of active coping behavior is different between LA and HA rats in escapable stress, but not of passive coping in inescapable stress, indicating that the internal anxiety level has certain correlation with the ability to cope with environmental challenge. Korte et al. [31] found that the anxiolytic action of the 5-HT 1A agonist ipsapirone in the burying test is restricted to active coping, which may implicate that serotonergic mechanisms differently underlie active and passive coping behaviors.

4.6. Neurochemical correlates and mechanisms

We have previously shown that male LA and HA Wistar rats differed in 5-HT tissue levels in the ventral striatum, but not in the neostriatum, frontal cortex, amygdala or ventral hippocampus. Furthermore, LA and HA rats did not differ with respect to norepinephrine or DA in these brain areas [51]. Although the role of other transmitter should not be ruled out [25,26,43,48], the previous serotonergic findings appear especially relevant for the present behavioral data, since considerable experimental evidence points at a relationship between anxiety and serotonergic activity in the brain [2,24,29]. Thus, it has been shown that decreasing serotonergic function has an anxiolytic effect in the elevated plus-maze [52] and can improve active avoidance performance [13,41]. In addition, the Wistar-derived Roman high and low-avoidance rats [10], which are bred based on the acquisition of active avoidance behavior, also differ with respect to plus-maze behavior, since high-avoidance rats showed higher levels of open arm activity and lower anxiety-related behavior, compared with low-avoidance rats [12,14,19]. These patterns were paralleled by increased 5-HT turnover in the cortico-limbic system of high-avoidance rats [11,9,34].

Unlike plus-maze, active avoidance and object-burying, the different levels of anxiety and of ventral striatal 5-HT [51] in LA vs. HA rats seem not to affect performance in the forced swim test. Previously, however, it was found that two rat lines [61] which differ in central 5-HT activity, differ in the forced swim test, but not in behaviors related to anxiety [22,39]. There, the serotonergic differences were more widespread (accumbens, prefrontal cortex, hippocampus, hypothalamus) than those of HA and LA rats, which indicates that different anatomical profiles of serotonergic activity may have substantial effects on behaviors related to anxiety and/or depression.

Furthermore, there may be a link to DAergic mechanisms, forced swim performance and locomotor behavior. First, we found here a relationship between changes of immobility time in the forced swim test and open field locomotion. Second, it is known that DAergic activity in cortical and striatal areas differs between low and high locomotor responders [42]. Third, fluoxetine, a 5-HT reuptake blocker, exhibits an antidepressant-like effect by reducing immobility in forced swim test, which was only observed in low locomotor responders [53]. Fourth, FSL rats, which have a deficit in forced swim behavior showed decreased extracellular DA levels in the nucleus accumbens, together with a blunted excitability by 5-HT [60]. Possibly, therefore, the interaction of transmitters like DA and 5-HT critically determine behavior in the forced swim test.

5. Conclusion

The present results suggest that individual differences of anxiety levels in the elevated plus-maze are predictive of behavioral reactivity in animal models of anxiety such as object burying and active avoidance paradigms, in contrast to the forced swim test, which is considered to be a model of depression. Based upon indirect evidence, we have suggested neurochemical systems that may contribute to these effects. Taking individual behavioral differences into account in psychopathological animal models is expected to improve our understanding of the neuronal mechanisms underlying anxiety and depression, and may help to further clarify the sometimes variable outcome of psychiatric drug testing.

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