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# Strain Differences in Open-Field and Elevated Plus-Maze Behavior of Rats Without and With Pretest Handling

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SCHMITT, U. AND C. HIEMKE. *Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling.* PHARMACOL BIOCHEM BEHAV **59**(4) 807–811, 1998.—Behavior of two rat strains was analyzed with and without 1-week pretest handling. Male rats (150–200 g body weight) of the strains PVG/OlaHsd (PVG) and Hsd:Sprague–DawleySD (SPRD) were tested once in a standard open field and an enriched open field and twice in an elevated plus-maze. Behavioral analysis revealed significant differences between the two strains and differential effects of the pretest handling procedure. SPRD rats displayed higher levels of activity and exploratory behavior than the PVG rats, whereas PVG rats were obviously less anxious. One-week pretest handling had an "anxiolytic" effect and changed activity and exploration-related behavior of the animals in both strains. Activity-related parameters were mainly affected in SPRD rats and anxiety-related ones in PVG rats. The data give evidence that differences in behavior of rats are not only determined genetically but also by preceding handling procedures. Because the two rat strains responded differentially to the pretest handling, we recommend to use a well-defined handling procedure before starting a behavioral test, especially when drug applications are included. © 1998 Elsevier Science Inc.

Rat Sprague Dawley PVG Open field Enriched open field Elevated plus-maze Handling

ETHOLOGICAL tests namely, the open-field and elevated plus-maze test, are used to analyze behavior that is based on natural conflict situations. In the case of the open field, it is the conflict between exploration of and the aversion against open, bright areas that determines animal behavior (2,10,33). It is used to analyze activity and anxiety profiles in rodents (1,33). The elevated plus-maze test, evaluated for rats (8,26–28) and mice (22), uses the openness combined with the elevation for generating behavioral changes (19,31). Both tests, but in particular the plus-maze, are standard tools to analyze the behavior for activity, exploration, and emotionality, and frequently used to screen drugs for their psychopharmacological potential, especially for anxiolytic or anxiogenic properties (9,30). They do not require time-consuming training and motivation, otherwise achieved by deprivation of the animals. Baseline behavior in the open field and elevated plus-maze, however, is determined by the individual animal itself and may be highly variable between individual rats (12). Moreover, recent publications on mice and rats give evidence that strain differences (11,13,20,32) as well as handling (12) are critical for the performance (14,20,21), and thus relevant for the baseline behavior.

The purpose of this study was to evaluate possible strain differences and the influence of handling on the "baseline" level in the two ethological tests for motor activity, anxiety, and exploration. We tested two rat strains, a standard albino strain (Spraque–Dawley) and a pigmented strain (PVG/OlaHsd) in a standard open field, an enriched open field, and an elevated plus-maze. The two rat strains were selected because different activity patterns have been reported (6) and differences in anxiety-related behavior were suggested from our own experience.

## METHOD

### *Animals*

Adult male rats (150–200 g) of the strain PVG/OlaHsd (PVG) and Hsd:Sprague–DawleySD rats (SPRD) were obtained from Harlan–Winkelmann (Borchen, Germany). Food and water were provided ad lib and a 12 L:12 D cycle was

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maintained (light on from 0600 h until 1800 h). Animals were housed four per cage at 22°C and a relative humidity of 60%.

*Experimental Design and Testing Routine*

A total of 32 rats, 16 of each strain, was devided into four groups of eight animals: group 1, PVG and group 2, SPRD, both without pretest handling, group 3, PVG, and group 4, SPRD, with 1-week pretest handling. Over a period of 7 days each rat was tested four times. Tests started on day 1 with the standard open field (10 min) and continued on day 3 with the enriched open field (10 min). On days 4 and 7 the rats were tested in the elevated plus-maze for 7.5 and 5 min, respectively. All tests were carried out between 0900 and 1300 h. Rats were transported within their home cages to the test room 1 h before starting the tests to minimize the influence of transportation stress. The test room temperature was 22°C. After each trial, the test arenas were carefully cleaned.

*Handling Procedure*

Animals of the handling groups received for 1 week, 5 min daily habituation handling. This procedure imitated situations that precede behavioral tests otherwise necessary for pharmacological treatments. Animals were taken out of the cage by the open hand and allowed to sit on the experimenter's hands and arm for 5 min to learn that they did not need to be afraid of being taken out of the cage. Finally, a strong grip in the neck was applied, as this is needed for drug injections.

These particular handling procedures ended the day before the first test. For all tests the animals were transferred on the open hand from the home cage to the arena. For the nonhandled animals this was the first contact with the experimentator.

*Instrumentation and Behavioral Tests*

*Open field.* The open-field test arena consisted of dark gray plastic with the bottom painted in ochre. It was divided into 25 squares (A1 to E5) by gray lines. The arena measured $100 \times 100 \times 35$ cm$^3$ and diffuse illumination came from two 100 W-lamps, 200 cm above the field.

The following data were recorded during the trials: total distance moved (cm), time moving (% of total recording time), time spent in outer zone (%), time spent in outer zone moving (%), time spent in inner zone (%), time spent in inner zone moving (%), time spent in center (%), latency to reach the center (s), number of rearing ($n$), and defecation ($n$).

*Enriched open field.* The above-described open-field arena was also used to test animal behavior in an enriched environment after introduction of two novel objects (a blue plastic duck of $\sim$9 cm and a green one of $\sim$14 cm diameter). The objects were placed on squares B2 and D3 of the arena. In the enriched open field, the following data were recorded: total distance moved (cm), time moving (% of total recording time), time spent in outer zone (%), time spent in outer zone moving (%), number of approaches to novel objects ($n$), number of rearings ($n$), and defecations ($n$). Object approach was defined by close movement to the object ($\geq$2 cm distance), usually the rats then inspected the object by sniffing.

*Elevated plus-maze.* The plus-maze was made of dark gray plastic. It consisted of two open arms $42.5 \times 15$ cm$^2$ and two enclosed arms $42.5 \times 15 \times 14$ cm$^3$. The arms extended from a central platform, $15 \times 15$ cm$^2$. The apparatus was connected to a metal frame on each end of the enclosed arms raising it 70 cm above the floor. The metal frame was carrying the video camera and the illumination lamps ($2 \times 100$ W). The data recorded for the elevated plus-maze were total distance moved (cm), total arm entries ($n$), time spent in open arm (% of total recording time), open arm entries (% of total arm entries), rearing ($n$), and defecation ($n$).

*Monitoring of Behavior*

For all tests the path of each rat was registered automatically by a computerized image analysis system. The hardware consisted of an IBM-type AT computer combined with a videodigitizer and a CCD video camera. The software used for data acquisition and analysis was EthoVision (Noldus Information Technology, Utrecht, The Netherlands). Defecation was quantitated by counting the numbers of fecal boli by the experimentator at the end of each trial. When an animal fell off the elevated plus-maze it was excluded from the analysis. This was the case once in both trials of group 1. Moreover, due to technical problems during the trials, group 1 in the open field and group 3 in the enriched open field had only seven complete records.

*Statistical Analyses*

A two-way factorial ANOVA/MANOVA was performed first. In the case of significance it was followed by a post hoc $t$-test [Tukey's honest significant difference (HSD) test for unequal $n$]. Differences were considered as significant for $p < 0.05$.

## RESULTS

Overall two-way MANOVA revealed significant strain [open field: $R(10, 18) = 12.75$, $p < 0.000003$; enriched open field $R(7, 21) = 27{,}46$, $p < 0.0000001$; elevated plus-maze $R(5, 23) = 19.36$, $p < 0.0000001$] as well as handling effects [open field: $R(10, 18) = 5.37$, $p < 0.001$; enriched open field: $R(7, 21) = 4.10$, $p < 0.006$; elevated plus-maze $R(5, 23) = 4.73$, $p < 0.004$], and on a significant interaction of strain and handling [open field: $R(10, 18) = 3.75$, $p < 0.007$; enriched open field: $R(7, 21) = 2.62$, $p < 0.04$; elevated plus-maze, $R(5, 23) = 2.70$, $p < 0.05$).

*Strain Effects*

SPRD rats were significantly more active than PVG rats. This was obvious for the parameters: total distances moved (Table 1 A; open field, $F(1, 27) = 131.75$, $p < 0.0000001$; enriched open field, $F(1, 27) = 82.00$, $p < 0.000001$; plus-maze, $F(1, 27) = 30.94$, $p < 0.000007$]; time moving [open field, $F(1, 27) = 97.44$, $p < 0.0000001$; enriched open field, $F(1, 27) = 50.48$, $p < 0.0000001$; and plus-maze: total arm entries, $F(1, 27) = 28.29$, $p < 0.00001$].

Exploration and emotional activity were also different between the two strains (Table 1 C). The activity in the outer and inner zone was always lower in PVG than in SPRD rats [time spent in outer zone and outer zone moving: open field, $F(1, 27) = 7.49$, $p < 0.01$, and $F(1, 27) = 93.87$, $p < 0.0000001$; enriched open field, $F(1, 27) = 10.12$, $p < 0.004$, and $F(1, 27) = 39.91$, $p < 0.000001$; time spend in inner zone and inner zone moving: open field, $F(1, 27) = 5.53$, $p < 0.03$, and $F(1, 27) = 17.50$, $p < 0.0003$]. The latency to reach the center field was significantly shorter in SPRD than in PVG rats ($F(1, 27) = 19.48$, $p < 0.0002$). Moreover, SPRD rats showed a higher frequency of object approaches in the enriched open field, $F(1, 27) = 25.64$, $p < 0.00003$. In contrast to the higher explorative activity of SPRD rats in the aforementioned tests, there was

TABLE 1

BEHAVIOR OF MALE RATS OF TWO DIFFERENT STRAINS WITH AND WITHOUT 1-WEEK PRETEST HANDLING IN AN OPEN FIELD (OF), AN ENRICHED OPEN FIELD (ENOF), AND AN ELEVATED PLUS-MAZE (PM)

| | PVG | | SPRD | | PVG + Handling | | SPRD + Handling | |
|---|---|---|---|---|---|---|---|---|
| Specific Effects | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| A Activity | | | | | | | | |
| Total dm OF (cm) | 1245.2* | 248.2 | 3727.4*§ | 173.8 | 1896.2† | 145.0 | 4744.2†§ | 321.5 |
| Total dm ENOF (cm) | 1542.3* | 172.7 | 3449.0*§ | 312.7 | 1594.0† | 199.7 | 4669.5†§ | 352.6 |
| Total dm PM (cm) | 781.8 | 120.7 | 1138.1 | 106.2 | 742.8† | 70.8 | 1451.6† | 83.3 |
| mv OF (%t) | 25.7* | 5.5 | 67.5* | 3.0 | 39.1† | 3.5 | 71.3† | 2.9 |
| mv ENOF (%t) | 42.8* | 3.9 | 69.8* | 3.8 | 39.8† | 5.1 | 70.8† | 3.7 |
| Total arm entries PM (*n*) | 4.7* | 0.8 | 12.6*§ | 1.9 | 10.5† | 1.6 | 18.4†§ | 1.3 |
| B Anxiety | | | | | | | | |
| InZ OF (%t) | 1.6 | 0.4 | 5.0 | 1.1 | 6.3 | 1.8 | 9.1 | 1.4 |
| InZ mv OF (%t) | 0.8 | 0.4 | 4.2§ | 1.0 | 3.3† | 0.9 | 8.3†§ | 1.4 |
| Ctr OF (%t) | 0.1 | 0.1 | 0.3 | 0.1 | 0.4† | 0.1 | 1.5† | 0.2 |
| Lat Ctr OF (s) | 596.5*§ | 3.5 | 368.7*§ | 75.1 | 346.7†§ | 65.2 | 100.7†§ | 28.9 |
| Obj. app. ENOF (*n*) | 11.6 | 2.4 | 24.3§ | 4.2 | 14.0† | 1.5 | 43.0†§ | 6.2 |
| Op arm entries PM (%) | 14.8§ | 7.6 | 22.3 | 4.3 | 43.8‡§ | 5.3 | 27.5‡ | 1.3 |
| Op arm (%t) | 1.8§ | 1.3 | 7.1 | 2.0 | 14.8§ | 3.5 | 14.4 | 2.1 |
| C Exploration | | | | | | | | |
| OuZ OF (%t) | 98.3 | 0.4 | 94.7§ | 1.2 | 93.3 | 1.3 | 98.4§ | 1.4 |
| OuZ ENOF (%t) | 90.1 | 1.8 | 84.2 | 2.7 | 87.2† | 1.1 | 75.0† | 4.2 |
| OuZ mv OF (%t) | 24.8* | 5.2 | 63.1* | 2.4 | 35.5† | 3.3 | 61.6† | 2.1 |
| OuZ mv ENOF (%t) | 35.5* | 3.3 | 56.9* | 2.7 | 30.0† | 4.7 | 48.6† | 1.6 |
| Rearings OF (*n*) | 10.6* | 2.0 | 21.9* | 2.0 | 10.6† | 1.5 | 16.3† | 1.8 |
| Rearings ENOF (*n*) | 10.4* | 1.6 | 22.5*§ | 3.8 | 7.9 | 1.8 | 11.1§ | 1.5 |
| Rearings PM (*n*) | 4.9 | 0.5 | 1.5 | 0.4 | 5.6 | 1.8 | 3.0 | 0.5 |

Values given are the means ± SEM of rats of the strains PVG and SPRD, respectively. Abbreviations: total distance moved (total dm), time moving (mv (% of observation time)), time spent in outer zone (OuZ), time spent in outer zone moving (OuZ mv), time spent in inner zone (InZ), time spent in inner zone moving (InZ mv), time spent in center zone (Ctr), latency to reach the center for the first time (Lat Ctr), novel object approaches (Obj. app.), open (op).

*$p < 0.05$ interstrain comparison nonhandled, †$p < 0.05$ interstrain comparison handled (‡$p < 0.06$), §$p < 0.05$ handled vs. nonhandled.

no difference to PVG rats in open-arm behavior (% open arm entries) in the elevated plus-maze test. The frequency of rearings was lower, $F(1, 27) = 21.35$, $p < 0.00009$, and the defecation rate always significantly higher in PVG than in SPRD rats ($p < 0.0000001$).

*Handling Effects*

The handling procedure influenced activity, exploration, and emotion related parameters (Table 1). The distance moved [open field, $F(1, 27) = 12.90$, $p < 0.001$; enriched open field, $F(1, 27) = 5.35$, $p < 0.03$], and the time the animals spent moving increased [open field, $F(1, 27) = 5.30$, $p < 0.03$]. In the elevated plus-maze the total distance moved remained unchanged but the number of total arm entries increased, $F(1, 27) = 15.11$, $p < 0.0006$.

With regard to exploration and emotional activity the time spent moving in the inner zone of the open field increased, $F(1, 27) = 10{,}38$, $p < 0.003$. In addition, the time spent in the center field increased and the latency to reach the center field decreased by the handling procedure, $F(1, 27) = 5.50$, $p < 0.03$, and $F(1, 27) = 23.27$, $p < 0.00005$. In the enriched open field the object exploration increased, $F(1, 27) = 6.60$, $p < 0.02$. Furthermore, in both tests the number of rearings decreased, $F(1, 27) = 5.30$, $p < 0.03$; $F(1, 27) = 8.33$, $p < 0.008$. In the elevated plus-maze test, handling significantly enhanced the following anxiety-related parameters: % open arm entries, $F(1, 27) = 13.29$, $p < 0.001$, and the time spent in open arms, $F(1, 27) = 17.50$, $p < 0.0003$.

*Second Trial Behavior in Elevated Plus-Maze*

For the 5 min second trial in the elevated plus-maze test significant effects of strain, $R(7, 21) = 20.94$, $p < 0.0000001$, and handling, $R(7, 21) = 6.90$, $p < 0.0003$, were also observed. Interaction of strain and handling, $R(7, 21) = 3.10$, $p < 0.02$, was also significant.

Strain differences were significant for distance moved, $F(7, 27) = 46.75$, $p < 0.0000001$, and total arm entries, $F(7, 27) = 10.10$, $p < 0.004$. Anxiety-related activity was similar. However, with respect to the handling effects all parameters were significantly different: [total distance moved $F(7, 27) = 25.37$, $p < 0.00004$; total arm entries, $F(7, 27) = 21.84$, $p < 0.00007$; rearing, $F((7, 27) = 22.48$, $p < 0.00006$; % open arm entries, $F(7, 27) = 16.73$, $p < 0.0004$, and latency to enter the open arms, $F(7, 27) = 43.00$, $p < 0.0000001$].

*Interaction of Strain and Handling Procedures*

Analysis of the specific effects revealed that the differences in activity pattern seen in both open-field tests were strain dependent and not influenced by the handling procedure. The activity levels differed between the strains in the elevated plus-maze but they reached statistical significance only after the handling procedure. Handling effects, exerting a sta-

tistically significant difference in activity-related behaviors, were only seen in the SPRD strain (Table 1 A).

In the case of exploration, the strain differences were not influenced by handling in the open-field tests (Table 1 C). SPRD rats showed a significantly higher outer zone activity and more rearings than PVG rats. The elevated plus-maze test showed only a trend to a higher rearing activity of PVG rats.

Emotion/anxiety-related parameters responded to the handling procedure with significant strain differences (Table 1 B). In the open field as well as in the plus-maze most handling effects were more pronounced on anxiety-related parameters in the PVG than in the SPRD rats (Table 1 B).

There was a trend indicating interstrain differences in activity without handling in trial 2 of the elevated plus-maze (Table 2). To reach significance the handling procedure was necessary. It increased the activity and lowered the anxiety state in nearly all cases.

## DISCUSSION

### *Strain Differences*

The present study revealed clear strain differences in behavioral tests of SPRD and PVG rats. Total distance moved and percentage of time moving in the test arenas as well as the number of total arm entries in the elevated plus-maze indicated higher motor activity levels of SPRD than of PVG rats. This was consistent with results of Asano (2), who also reported a high activity level in SPRD rats compared to Wistar rats and confirmed observations of Brett and Pratt (6), who reported that PVG-hooded rats are less active than SPRD rats (6).

Behavior also differed in parameters related to exploration and emotion or anxiety. The behavioral profile displayed by the SPRD rats consisted of a high level of explorative activity, as indicated by the time spent in the distinct maze zones and the amount of vertical and horizontal activity [see also (15)]. Under the influence of habituation handling these results are attenuated confirming earlier results (15). With regard to anxiety, the interpretation of the results is more difficult, because anxiety effects are confounded by locomotor activity (12). SPRD rats outperformed PVG in their locomotor activity in the open-field arena and elevated plus-maze. However, significant strain differences were not observed for open-arm activities in the plus-maze (open arm entries, time spent on open arm). We suggest that this discrepancy might be explained by a higher anxiety level in SPRD rats. Considering for open-field behavior the parameters time spent in the outer zone and the outer zone movement together with rearings as fear-related behavior (20,24) and the number of rearings in the plus-maze as exploration (20,29), PVG rats seemed likely to be less anxious than SPRD rats. Handling intensified this difference. It must be mentioned, however, that the suggested strain differences in anxiety-related behavior may also be due to different emotional reactions (15). Center activity in the open field and novel object exploration, which are indicators of emotionality (15), scaled higher in SPRD than in PVG animals.

Another relevant factor that might underlie the strain differences is the emotional memory. For mice and also rats it has been shown that an individual's reaction towards an anxiogenic stimulus can be changed by cognitive processes (3,5,17). Strain differences in anxiety may, therefore, not only reflect a different genetic background but also different emotional memories (10,11,15,25). This is also supported by interstrain differences reported by investigators who studied differentially bred rat lines (3,14,15,20).

### *Handling Effects*

The present study showed that pretest handling markedly affected the behavioral pattern of animals. The data supported and extended results of Fernandez-Teruel and colleagues (14,15), who showed that handling affects behavior and drug responses. Their and our habituation handling had an anxiolytic effect similar to benzodiazepine-induced behavioral changes (4,7,17). Activity-related behavior was mainly influenced in the SPRD strain (total distance moved, total arm entries). Anxiety-related parameters were primarily affected in the PVG strain (open arm entries, time spent in open arms). This differential response might be due to a different genetic background of the two animal strains. However, postnatal influences also determine in part the emotional reactivity and lead to different emotional baselines in adulthood (15,16). Exploration activity of psychogenetical selected rat lines is increased, whereas emotional reactivity is decreased after postnatal handling. But the effect is more pronounced in the low avoidance line (15,16).

TABLE 2

BEHAVIOR OF MALE RATS OF TWO DIFFERENT RAT STRAINS WITH AND WITHOUT 1-WEEK PRETEST HANDLING DURING A SECOND TRIAL IN AN ELEVATED PLUS-MAZE

| | PVG | | SPRD | | PVG + Handling | | SPRD + Handling | |
|---|---|---|---|---|---|---|---|---|
| Special Effects | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM | Mean | ±SEM |
| Activity | | | | | | | | |
| Total dm (cm) | 183.7*‡ | 19.5 | 634.0*‡ | 82.7 | 500.2†‡ | 55.9 | 1066.3†‡ | 101.6 |
| Total arm entries (*n*) | 1.4 | 0.5 | 5.5§ | 1.2 | 8.3† | 1.2 | 15.9†‡ | 3.1 |
| Anxiety | | | | | | | | |
| Op arm entries (%) | 7.1‡ | 7.1 | 6.3§ | 4.1 | 40.6‡ | 8.6 | 31.0‡ | 4.7 |
| Op arm (%t) | 1.0‡ | 0.7 | 2.2 | 1.2 | 25.2‡ | 7.2 | 10.4 | 2.1 |
| Rearings | 0.1‡ | 0.1 | 0.4 | 0.3 | 5.3‡ | 0.9 | 3.3 | 1.3 |

Values given are the means ± SEM of rats of the strains PVG and SPRD, respectively. The second trial was done 3 days after the first one. Abbreviations: total distance moved (total dm), time spent in open arms (op arm (% of observation time)).

*$p < 0.05$ interstrain comparison nonhandled, †$p < 0.05$ interstrain comparison handled, ‡$p < 0.05$ handled vs. nonhandled.

*Second Trial Behavior in Elevated Plus-Maze*

Recent publications that had measured anxiety with the plus-maze test reported differences between the first and later trials (3,19,23). This has consequences for the release of neurotransmitters and responses to pharmacological treatments, for example, benzodiazepines (18,21). A second trial in the present experiment lead to a decrease in activity and anxiety-related parameters. However, our handling procedure diminished the decrease in both rat strains. This indicates that the handling had a similar effect as a first trial experience on the elevated plus-maze (19), thus changing the emotional memory.

As mentioned above, ethological tests are useful to evaluate motor activity and emotional behavior in rodents. Users of these tests, however, must give attention to genetically and environmentally determined baseline behavior (12,13). Our data support the view that a series of tests and not a single test should be used to characterize animal behavior (14). In addition, we recommend to use a strict pretest handling procedure to obtain well-defined control behavior, especially when handling is necessary for a pharmacological treatment.

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