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The Effect of Stressor Level Grading on the Stimulus Seeking Behavior of Rats Differing in Emotional Reactivity[1]

A natural disaster – such as a flood – is a sequence of events: swollen water level leading to the flooding of homesteads – primary stressor and later environmental consequences – secondary stressor syndrome. In order to be valid, an experimental model must ensure similarity of the stress-evoked behavioral symptoms. The most frequently administered behavioral tests measure exploratory behavior in the broad sense (we used the following test battery: self-exposition chamber, open field and elevated cross-maze). We also included emotional reactivity in the experimental design in order to test the idea that lower emotional reactivity alleviates the consequences of stress and therefore acts preventively. Reduced emotional reactivity and increased stressor intensity additively contribute to increased exploratory behavior. A main handling effect is found for most indices of emotional behavior. The proposed experimental model seems to meet two important criteria: it has face validity and it evokes very clear behavioral consequences, ones which are universal for most indices of exploratory behavior.

Keywords: *stress, emotional reactivity, exploration, experimental model*

Introduction

In psychological research on human beings the experimenter is often confronted with various restrictions (ethical, methodological) which make it difficult or sometimes even impossible to test his or her research hypotheses. When this happens, experimental animal models imitating both the stimulus situation and its consequences may come to rescue.

The experimental study of stress is one of those areas where experimenters are especially willing to resort to animal studies (Farley, Matysiak and Trojan, 2006). It is not easy to develop a good animal model, however, and it often needs laborious and time-consuming investigations.

The idea to create a laboratory model imitating a natural disaster and its consequences was inspired by the results of an earlier study (Trojan, Farley & Matysiak, unpublished data). This study adopted a number of stressors directly emulating the environmental consequences of a cataclysm – reduced life space, limited access to food and disruption of the circadian light/dark cycle. A natural disaster, however, for example a flood, is a whole sequence of events: water swells and floods homesteads (primary stressor) leading to environmental consequences (secondary stressors). Therefore, in order to ensure at least face validity (Adamec et al., 1998; Willner, 1993) of the model, exposition of secondary stressors must be preceded by a procedure imitating flooding.

An important criterion of validity of the experimental model is similarity of the behavioral symptoms evoked by the experience of stress. The most common behavioral tests measure stimulus seeking behavior in general and exploratory behavior in particular.

According to the widely accepted interpretation of exploratory behavior its purpose is to gather information about the environment (Archer & Birke, 1983; Renner, 1988, 1990; Matysiak, 1992, 1993). The exploring organism obtains information of fundamental biological significance – information about possible hazards (the presence of enemies, predators), about competition (the presence of other members of the same species), about sources and availability of food and also about the effects of its exploratory behavior on the environment. Exploratory activity is therefore an excellent indicator of an organism's

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adaptive capacity. Every change in the stimulus field, even in an otherwise familiar environment, instigates the organism to explore (Matysiak, 1993).

Many writers have underscored the role which emotions play in the motivational mechanisms of exploratory behavior. According to Wells, Williams and Lowe (1971), it is widely accepted that the so-called handling procedure differentially affects a whole range of behavioral and physiological signs. Levine et al. (1967) demonstrated, for example, that handled rats mature earlier, grow faster, are more resistant to stress and are more willing to explore an unfamiliar environment. On the other hand, handled animals are much less reactive emotionally (Denenberg, 1964; Ardila, Rezk, Polanco & Pereira, 1977). In other words, handling allows us to obtain a group of animals which differ in many ways from animals which have not been handled. Such alterations in animal activity are usually interpreted as a sign of reduced emotional reactivity. This interpretation has been supported empirically. Several studies (e.g., Núňez, Ferré, Escorihuela, Tobena & Fernández-Teruel, 1996; Vallée et al., 1997) have demonstrated that handling reduces corticosterone secretion in stressful situations and also reduces the activity of the hypothalamic-pituitary-adrenal system.

Taking these findings as our point of departure we made an attempt to include emotional reactivity in our experimental design testing the hypothesis that reduced emotional reactivity alleviates the consequences of stress and therefore plays a preventive role.

Method

Research objects

The experiment was run on 128 (64 female and 64 male) inbred Lewis rats. The animals were kept in artificial day/night conditions with a 12-hour illumination cycle (28 lx.) in house cages (28x34x54 cm), eight rats per cage. Temperature was held constant (22^{o}C). The animals had unlimited access to food and water.

Apparatus

Three different types of apparatus were used: the open field, the elevated cross-maze and the self-exposition chamber.

The open field was a lidless box whose walls each measured 75 cm and whose height was 60 cm. The box was made of laminated fibreboard. The floor and all the walls were white and were divided into 25 identical squares with a black grid of lines 1 cm thick. During testing the open field was illuminated with a light of 200 lx intensity. The following parameters were recorded: time spent in the central part of the field (the 9 central squares), ambulation, number of rearings, number of climbings, and number of defecations and urinations. The test session was filmed with a video camera.

The elevated cross-maze had two arms, both of which were 100 cm long. The arms crossed in the middle at right angles. One arm was additionally sheltered with a band 50 cm high to provide a “safety zone”. The apparatus was made of white laminated fibreboard and was mounted on a stand, 70 cm above floor level. Light intensity during testing was 300 lx. The following parameters were measured: latency, number of entries onto the safe arm, number of entries onto the unsafe arm, number of alternations, number of defecations and urinations. The test session was filmed with a video camera.

The self-exposure chamber was a 33x30x27 cm box made of plexiglass and aluminium. The floor was made of metal bars, 0.5 cm in diameter, mounted every 1.7 cm. Each of the side aluminium walls of the chamber had a hole, 3 cm in diameter, situated 10 cm above floor level. Whenever the rat put its head into one of the holes a photocell registered the event. If the rat put its head into one of the holes (the experimental one) a 27 lx light switched on for 3 seconds. If it put its head into the other (control) hole, nothing happened. The number of head-dipping reactions to each hole and the number of alternations between holes were registered. The session was controlled and monitored by computer software.

Procedure

When the rats were 28 days old they were transported to the laboratory and put into the house cages, 8 per cage. Every day for 30 days, beginning with day 21 of their stay in our laboratory, a randomly selected half of the rats were submitted to the handling procedure. Each animal was taken out of its cage, placed on the experimenter’s forearm and stroked for one minute then put into an empty cage. The remaining seven rats from the same house cage were treated in the same way.

Next, both groups – the handled one and the nonhandled one – were divided into four subgroups each, 16 rats per subgroup. Three of the subgroups were exposed to stressors of varied intensity: (1) forced swimming test (FST), (2) secondary stressors set (SSS) and (3) a combination of the two previous manipulations (FST + SSS). The fourth, control, group (C), was not stressed in any way. Hence there were eight experimental subgroups (see Table 1). Sex is not included in the grid because it was not analysed.

In the forced swimming procedure the rats were submerged in an aquarium filled with water (20^{o} C) where they had to keep on the surface of the water for 3 minutes.

Application of the secondary stressors set involved transferring the rats from their present conditions to conditions inferior to the ones the animals had been accustomed to. The animals were put into smaller steel cages measuring 21x28x45 cm, eight per cage (i.e., more

Table 1
Division of animals into experimental groups (sample N=128).

No handling (N=64)				Handling (N=64)			
FST (N=16)	SSS (N=16)	FST+SSS (N=16)	C (N=16)	FST (N=16)	SSS (N=16)	FST+SSS (N=16)	C (N=16)

crowded conditions). Crowding is a frequent way of stressing rodents experimentally (Koolhas & Bohus, 1995; Kirrilov et al., 2000; Dronjak et al., 2004;). Access to food was also reduced – the animals were fed only twice a week (Leal et al., 1995; Nazarloo et al., 2002; Ekimowa, 2005). Additionally, the room was lit 24 hours a day with an intensity of 120 lx (Vanderschuren et al., 1995). The rats remained in these adverse conditions until the end of the experiment. The stressful manipulations we used are typical for studies of chronic stress (Hendley, 2000; Rossler et al., 2000).

The behavioral tests were conducted in the following order: the open field (day one), the elevated cross-maze (day two) and the self-exposition chamber (day three). The open field test lasted 3 minutes from the moment the rat was placed in the centre of the field. The elevated cross-maze test also lasted three minutes from the moment the animal was placed at the intersection of the arms. The self-exposition chamber test lasted 30 minutes.

Results

The experimental design was a two-way 2x4 analysis of variance (handling and stressors).

Exploratory behavior

The following measures of exploratory behavior were submitted to statistical analysis: in the self-exposition chamber – (1) total exploratory behavior (i.e., the number of times the rats put their heads into both holes) and (2) number of alternations in consecutive choices between holes; in the open field test – (3) ambulation, (4) number of climbings and (5) number of rearings and in the elevated cross-maze – (6) number of entries into both arms of the maze.

In the self-exposition chamber level of alternation (2) was significantly affected by both handling, $F(1,119)=6.556$, $p=0.012$ and stressors, $F(3,119)=5.820$, $p=0.001$. Handled animals have a higher alternation level compared with nonhandled animals and the differences between the various stressor levels (see Fig. 1) are as follows: the control (C) group and the FST group score higher on alternation than both the SSS and the SSS+FST groups – $p=0.003$ and $p=0.006$ respectively. The differences in general activity (1) are not significant.

Statistical analysis of ambulation (3) in the open field revealed a main effect for handling, $F(1,119)=9.064$,

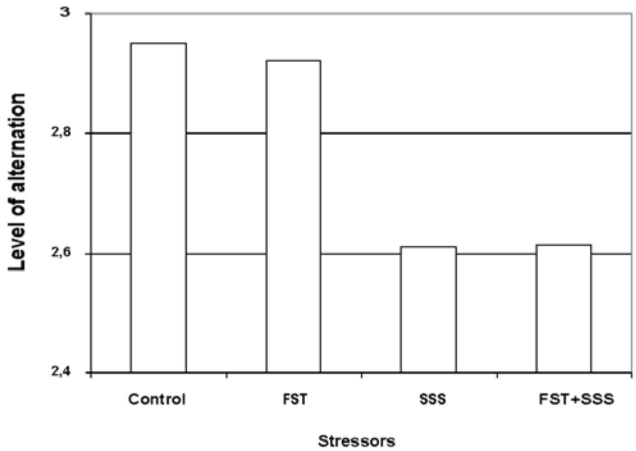

Figure 1. Level of alternation in groups submitted to stressors of various intensity (control – control group, FST - forced swimming test group, SSS - secondary stressors set, FST + SSS – disaster group).

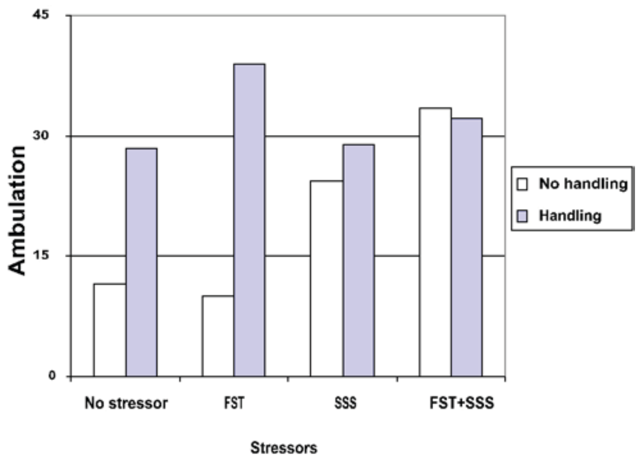

Figure 2. Level of ambulation in handled and nonhandled groups submitted to stressors of various intensity (control – control group, FST - forced swimming test group, SSS - secondary stressors set, FST + SSS – disaster group).

$p=0.003$ (handled rats were more active than nonhandled rats) and also an interactive effect of the experimental factors, $F(1,119)=2.697$, $p=0.049$. Analysis of simple effects showed that the control group and the nonhandled FST group had significantly lower ambulation levels than all the handled groups whereas the nonhandled FST+SSS group had an intermediate position but did not differ significantly from either of the remaining groups (*p* levels ranged from 0.001 to 0.04). Figure 2 shows the mean ambulation scores for handled and nonhandled rats submitted to various degrees of stress.

A similar pattern of results was obtained for climbing (4) where we found a main effect of handling, $F(1,119)=26.755$, $p=0.001$ (handled animals were more

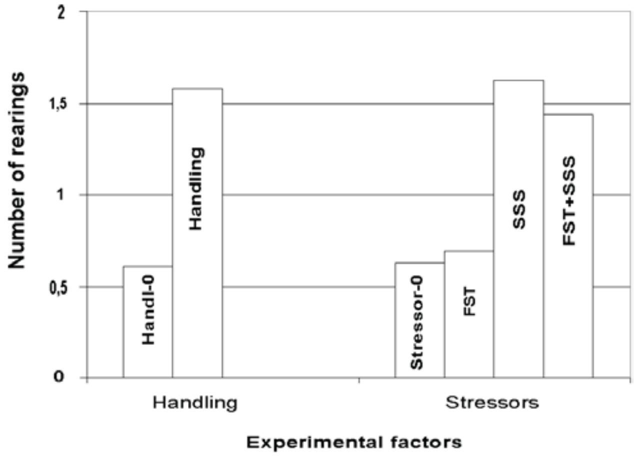

Figure 3. Additive effect of experimental factors on the number of rearings in the open field (FST - forced swimming test group, SSS - secondary stressors set, FST + SSS – disaster group).

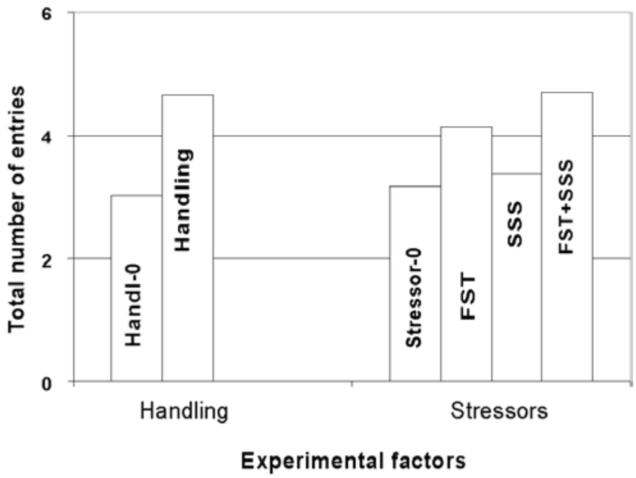

Figure 4. Additive effect of experimental factors on the total number of entries onto the elevated cross-maze (FST - forced swimming test group, SSS - secondary stressors set, FST + SSS – disaster group).

active than nonhandled animals) and an interactive effect, F(3,119)=3.728, p=0.013.

A different pattern emerged for rearing (5). Here significant main effects but no interactive effects of the experimental factors was found (Fig. 3): handling – F(1,119)=11.425, p=0.001 (handled animals had more rearings than nonhandled animals) and stressors – F(3,119)=3.160, p=0.027. The differences for the stressor factor, displayed in Figure 3, take on the following pattern: the difference between the control group and the FST group are not significant and neither is the difference between the SSS group and the FST+SSS group. Both these pairs, on the other hand, differ significantly (*p* values range from 0.016 to 0.047).

Exploration in the elevated cross-maze, i.e., total number of entries onto both arms (6) and rearing in the open field (5) are produced by the additive effect of the two experimental factors – handling (handled rats are more active than nonhandled rats) and stressors, F(1,119)=18.886, p=0.001 and F(1,119)=3.452, p=0.019 respectively (Fig. 4).

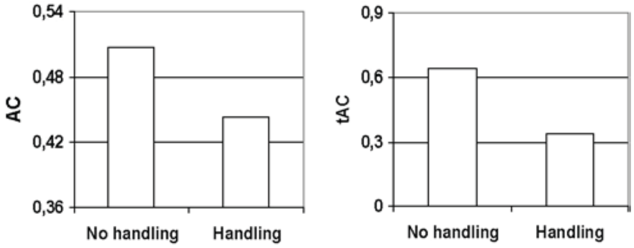

Figure 5. Effect of handling on the indicators of anxiety in the elevated cross-maze (AC - the anxiety coefficient for the number of entries, and tAC - for time spent on both arms.

As far as stressors are concerned, the difference between the control group and the SSS group is not significant and neither is the difference between the FST and FST+ZWS groups. On the other hand, the difference between the two pairs is statistically significant (*p* levels range from 0.005 to 0.015).

Emotional behavior

The following indicators of emotional behavior were analysed: in the open field test – (7) latency, (8) ambulation in the central area of the field, (9) number of defecations and (10) number of urinations, in the elevated cross-maze – (11) latency, (12) number of entries into the low-anxiety arm (Al), (13) number of entries into the high-anxiety arm (Ah), (14) time spent on the low-anxiety arm (tAl), (15) time spent on the high-anxiety arm (tAh) and (16) the anxiety coefficient for the number of entries – AC=Al/Al+Ah, and (17) for time spent on both arms – tAC=tAl/tAl+Ah.

Two-way analysis of variance for indicator (8), i.e., ambulation in the central area of the field, revealed a main effect for handling, F(1,119)=8.687, p=0.004 (handled animals scored higher than nonhandled animals) whereas indicator (10), i.e., number of urinations, was significantly affected by the interaction of the experimental factors – F(3,120)=3.008, p=0.003 but no significant main effects emerged. Simple effects analysis showed that the control group, the handled FST group and the nonhandled SSS group had a significantly higher urination score than the handled SSS group and the handled FST+SSS group whereas the nonhandled FST group, the handled stress-free group and the nonhandled FST+SSS group had an intermediate position and did not differ significantly from any of the remaining groups (*p* levels ranged from 0.007 to 0.05). The differences for latency (7) and number of defecations (9) are nonsignificant.

Like all the indicators discussed above, the indicators of behavior on the elevated cross-maze were submitted to two-way analysis of variance. As far as number of entries onto the low-anxiety arm (12) are concerned, two main effects emerged – handling, F(1,119)=4.664, p=0.003 and stressors, F(3,119), 4.742, p=0.004 (handled rats had higher scores than nonhandled rats and group FST+SSS had higher scores than the control group, p=0.003). Only one factor significantly affected the number of entries onto the high-anxiety arm, i.e., handling, $F(1,119)=24.366$, $p<0.001$ (handled rats entered the high-anxiety arm more frequently than nonhandled rats). Only handling had a significant effect on time spent on the low-anxiety arm (14) and the high-anxiety arm (15), $F(1,119)=40.201$, $p=0.001$ and $F(1,119)=45.786$, $p=0.001$ respectively (nonhandled animals spent more time on the low-anxiety arm than handled animals and the reverse pattern emerged for the high-anxiety arm). Analysis of anxiety indicators AC (16) and tAC (17) also revealed a main effect of handling only (Fig. 5) – $F(1,119)=10715$, $p<0.001$ and $F(1,119)=46.576$, $p<0.001$ respectively (in both cases handled rats score lower than nonhandled rats). The differences for latency (11) were nonsignificant.

Discussion

Grading stressor intensity, from complete lack of stressors through FST, SSS to the stressor imitating a disaster (FST+SSS), basically leads to increased exploration with the increase in stressor complexity, and hence stressor intensity. The only exception is alternation in the self-exposition chamber where a reverse pattern emerged – the more intense the stressors the lower the level of alternation. This indicator, as opposed to other indicators of exploration has been interpreted (Matysiak & Osiński, 2003) as an expression of novelty seeking rather than propensity for exploration in general. Hence the present finding supports the hypothesis signalled earlier (Matysiak, 1999) that there are two different motivational systems, one which regulates behavior towards novel stimuli and one which regulates the general level of exploratory activity.

Ambulation in the open field is a sign of exploration in the most general sense. The finding showing the effect of interaction between the experimental factors on level of ambulation exactly corroborates the findings of similar former research (Trojan, Farley & Matysiak, unpublished data). The results shown in Figure 2 suggest that level of ambulation in the nonhandled groups increases gradually to achieve its maximal value in group FST+SSS whereas in the handled groups, whatever the intensity of the stressor, the level of ambulation is invariably high and more or less constant. An almost identical pattern emerged for climbing which is not surprising considering that the correlation between ambulation and climbing scores is $r=0.738$. Handling acts as nonspecific stimulating training which improves the organism's adaptive capacity and therefore helps it to cope with stress better (Clausing et al., 1997).

In most cases, however, exploratory activity is produced by the additive effect of the two experimental factors. Handling, which permanently reduces emotional reactivity, helps to increase the level of exploratory behavior, just as increasing stressor intensity does.

With the exception of entries onto the low-anxiety arm, emotional behavior is not affected by the stressor factor whereas the effect of the handling factor emerged for all the indicators of emotional behavior. This supports the idea reported in the literature time and time again that handling permanently modifies the level of emotional reactivity.

All stressed animals, whatever the level of stressor, produced more exploratory behavior than the control group of animals. As predicted, the FST effect is the most moderate and unstable one (it changes depending on type of exploration). The SSS effect is comparable to the FST+SSS effect but also tends to be unstable, just like the FST effect.

The suggested experimental model imitating a disaster apparently meets two important criteria: it has face validity and it evokes the clearest behavioral consequences, ones which are universal for most indicators of exploratory activity.

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