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Response to novelty of various types in laboratory rats

Wojciech Pisula[1,2], Rafał Stryjek[1], and Aleksandra Nałęcz-Tolak[1]

[1]The Warsaw School of Social Psychology, and [2]Institute of Psychology, Polish Academy of Sciences, Chodakowska 19/31, 03-815 Warsaw, Poland

Abstract. One purpose of the two studies reported here was to examine the reactions of rats to changes in the spatial arrangement of a familiarized environment under low stress conditions. The second purpose was to test the role of rats' experience with novelty. In Experiment I the novelty was manipulated by introducing new tunnels into one zone of the experimental chamber. The introduction of novelty took place after 11 habituation sessions. In Experiment II in the course of habituation sessions the experimental group of rats was subjected to a persistent change of tunnel arrangement in the experimental zone, whereas for the control group nothing changed. All animals reacted to the novelty with increased time spent in the experimental zone and decreased time spent in other zones. Both experiments show that under low stress conditions rats demonstrate a positive response toward novelty and that their previous experience with novelty does not affect that reaction.

The correspondence should be addressed to W. Pisula, Email: Wojciech.Pisula@wp.pl

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INTRODUCTION

Various organisms tend to cope with environmental change in different ways. A highly novel or biologically significant stimulus (predator-like patterns) usually evoke stereotypical responses, called Species Specific Defense Responses (SSDRs) such as freezing, tail separation (lizards), burying, spreading a putrid odor or fleeing. For an extensive review see Fanselow and De Oca (1998).

By comparison, reactions to novelty under low stress conditions are less apparent and rather poorly understood. Nevertheless, they are no less important, since in many species they constitute a large part of the behavioral repertoire. Rats are known for their avoidance of novel objects in natural conditions (Barnett 1963, Calhoun 1963). This phenomenon also appears in the responses to changes in locations of familiar objects. Most manipulations in studies of animals' responses to novelty have involved spatial rearrangement of existing physical objects or the introduction of novel objects (e.g. Calhoun 1963, Gouteux, et al. 1999, Picq and Dhenain 1998, Pisula and Siegel 2005, Renner and Seltzer 1994).

Recent data (Pisula 2003, Pisula and Siegel 2005) show that under low-stress conditions the introduction of novel objects, removal of these objects, and the spatial rearrangement of familiar ones evoke positive responses toward the source of novelty. In these studies, rats were allowed to explore a familiar cage, and non-emotogenic changes were introduced after a long multi-trial habituation phase. Rats pre-trained in a procedure that eliminated emotional arousal oriented their behavior toward the source of novelty, either an object introduced into their space or changed patterns displayed on a computer screen. This positive response was associated with a lack of behavioral signs of emotional arousal, such as grooming, freezing, immobility, defecation or stretching. Therefore, it is proposed that behavioral responses to emerging novelty observed in these studies are specific to the rat's novelty-related behavior, free from emotional disturbance.

It was found (Pisula and Osiński 2000) that Roman Low-Avoidance (RLA/Verh) and Roman High-Avoidance (RHA/Verh) rats differed in both quantitative and qualitative aspects of exploratory behavior. Also under our testing conditions males of both sub lines were found to be more exploratory than females, which contradicted some traditional views (Escorihuela et al. 1997, Fernández-Teruel et al. 1991, 1994, Gray 1971, Hughes 1968). RHA/Verh rats showed a less diverse but more exploratory repertoire than did RLA/Verh rate. Males of both sub-lines showed more behavioral sequences than females. These notable differences were found under novel low-stress conditions, leading to the conclusion that emotional reactivity may have profound effects on behavioral regulation which are not limited to high intensity stimulation. The novelty related behavior has been tested extensively in several strains of rats. However, some of them have been investigated especially often (Matysiak et al. 1992, Ostaszewski and Pisula 1994, Ostaszewski et al. 1992). For that reason we decided to use these well-known strains of rats (WAG, LEWIS, and BN/Han) in the first reported in this paper study.

In the present studies, the emotional response was purposefully reduced by a procedure of repetitive placement in the experimental chamber. This procedure proved to be effective with AUGUST and Roman rats in earlier research (Pisula 2003, 2004, Pisula and Siegel 2005). In addition, all rats were systematically handled following arrival in our laboratory, as handling was found to reduce emotional reactivity (Pisula et al. 1992).

The present study, involving two experiments, was a follow up on our previous work, with the main goal of investigating the persistence of the novelty-related behavior in non stressful conditions. Habituation of the response to novel objects or to object rearrangement is context dependent. If the novel object is placed in a familiarized environment, the initial response in animals is very clearly directed toward the source of change (Pisula 2003). Subsequently habituation of this response takes place. On the other hand, the level of environment complexity affects its attractiveness. Rats (and many other animals) prefer an environment with a large number of elements (Berlyne et al. 1966). Therefore, the change of behavior obtained in studies based on the introduction of novel objects may be to some extent a reflection of the shift of their preferences for complexity, and not merely a response to novelty. In the present study, the course of novelty-evoked response habituation was selected as the measure of the novelty effect. The persistence of the behavioral change would stand as evidence of the change of subjects' preferences related to the complexity of the environmental configuration. Since opportunity to experience various levels of complexity and intensity of stimulation, may play an important role in regulating the adaptation to novelty, the purpose of the second experiment was to evaluate the influence of the experience of continuous environmental change on the response to novelty.

EXPERIMENT I

Methods

SUBJECTS

WAG (WISTAR ALBINO GLAXO), LEWIS, and BN/Han rats were obtained from the Medical Research Center of the Polish Academy of Sciences in Warsaw. They arrived in our laboratory at the age of seven weeks. Thirty subjects (five females and males of each strain), about 90 days of age at the onset of the experiment were used. They were handled mildly daily by being tickled and being carried by human hand. Rats were housed in transparent plastic (polycarbonate) cages (610L, 415W, 215H mm, floor area 2530 cm^2), four subjects per cage. The housing conditions fulfilled the criteria set by the local and European Ethics Commission.

APPARATUS

The box used for exploratory behavior measured 83.5L × 57.5W × 80H cm, and is shown in Fig 1. It was illuminated with a red light of low intensity (50 lx).

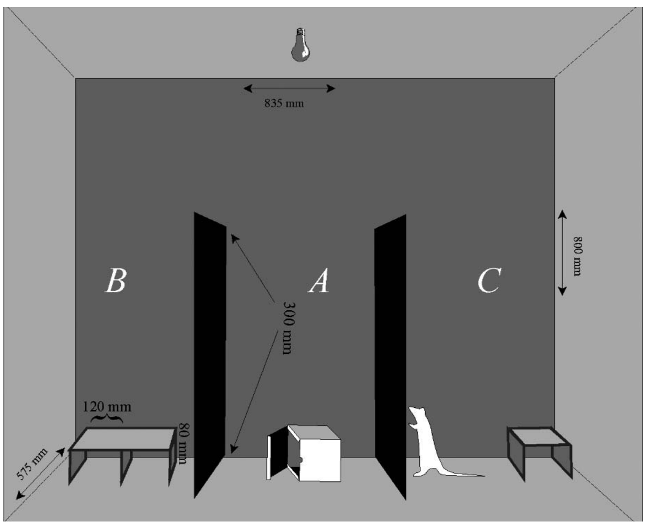

Fig. 1. Experimental cage used in this study. (A) start zone (the box seen in this zone was used as a rat transporter); (B) control tunnel zone; (C) experimental tunnel zone.

PROCEDURE

In order to control the level of novelty, a procedure of habituation to the experimental chamber was used. Every day, from the beginning of the experiment onward, each animal was placed in the experimental chamber for a six minute period. The experimenter would leave the experimental room immediately after placing the container with the rat in the chamber. The first eleven sessions were the habituation sessions, with the chamber in a standard mode (see Fig. 2). Experimental manipulation consisting of the introduction of the novelty (new tunnels in zone "C") took place on the twelfth session. The subsequent eight sessions were conducted with the chamber remaining in the experimental mode. Figure 2 shows the details of the experimental situation.

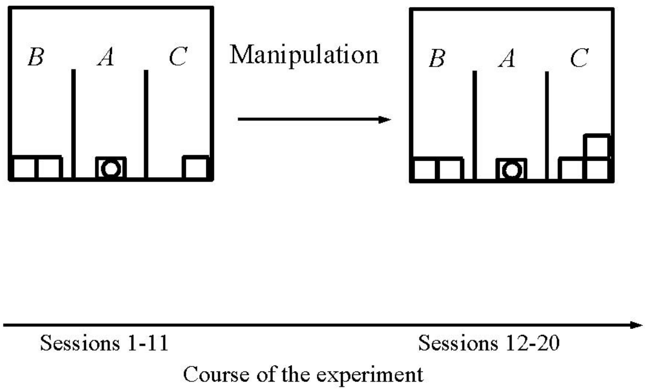

Fig. 2. Experimental conditions. Sessions 10 through 13, 16, and sessions 19 and 20 were video recorded. All the tunnels showed in the chamber could be entered by rats.

Sessions 10 through 13, 15, 19, and 20 were video recorded. The experimental room was dark. The video camera was shown not to generate noise, including ultrasound frequencies, which could affect the animal. It was placed approximately 2m from the front cover of the experimental chamber. The front cover was made of transparent Plexiglas. For the purpose of analysis, the following behavioral activities were measured: walking, floor sniffing, object contacts, time spent in the given zones, entering the tunnels. The onsets of all activities (including walking) were counted as separate behavior episodes. All six-minute sessions were divided into two three-minute intervals for analysis. A three-factor MANOVA, involving $2_{(sex)}$ × $3_{(strain)}$ × $14_{(3\text{-min interval})}$ was performed.

Table I

The results of the MANOVA analysis

Variable	Effect	*F*	*DF*	*P*
Duration of staying in the experimental tunnel zone	trials strain by trials	26.59 1.98	13, 286 26, 286	0.001 0.01
Duration of staying in the start zone	trials strain by trials	10.83 1.75	13, 286 26, 286	0.001 0.05
Duration of staying in the control tunnel zone	trials strain by trials	17.34 1.63	13, 286 26, 286	0.001 0.05
Duration of staying inside the experimental tunnels	trials strain by trials strain by sex by trials	11.94 2.85 1.99	13, 273 26, 273 26, 273	0.001 0.001 0.05
Walking (onsets)	trials strain by trials sex by trials	12.63 2.76 2.17	13, 286 26, 286 13, 286	0.001 0.001 0.05

Results

Table I shows the statistical effects obtained in the analysis.

The experimental design applied in this study was based on repeated measures, and therefore our main focus is on the interactions between strain, sex, and trial effects.

Results may be descriptively summarized in terms of immediate response to novelty and its subsequent habituation. The experimental manipulation of introducing the novelty resulted in increased time spent in the experimental tunnel zone (Note: This has not been defined or described in the text) by all subjects. The BN rats showed more limited immediate response (session 12th) than their WAG and LEWIS counterparts (Fig. 3). It is also noteworthy that the behavioral change persisted from the twelfth throughout the twentieth session.

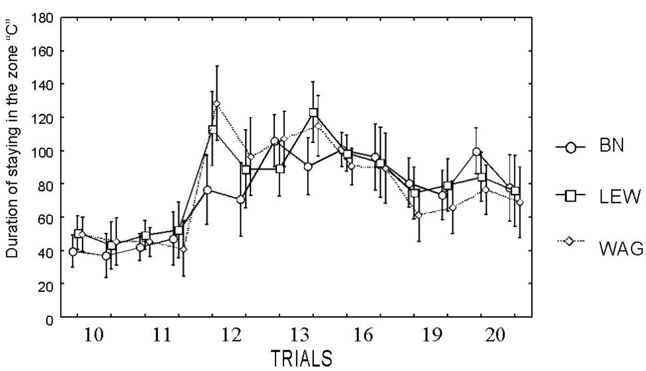

Fig. 3. Duration of time that rats spent in the experimental tunnel zone over recorded sessions. Each bar represents the three minute period of measurement within a given trial.

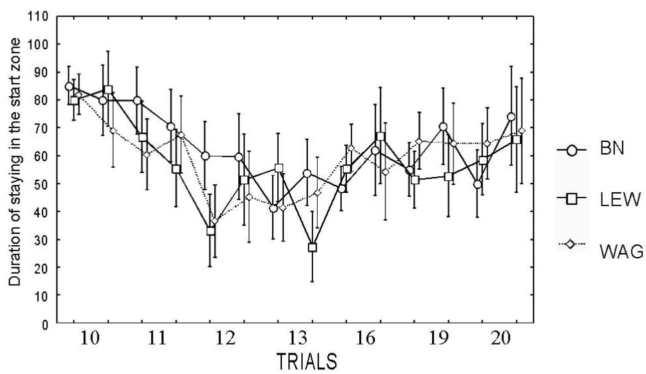

Fig. 4. Duration of time that rats spent in the start zone over recorded sessions. Each bar represents the three minute period of measurement within a given trial.

All subjects responded to the experimental manipulation with a decrease in time spent in the start zone, with WAG and LEW rats showing a greater decrease than BN rats (Fig. 4). This general effect tended to fade during sessions 19 and 20.

All rats spent less time in the control tunnel zone (note described) after introduction of the novelty into the chamber. The WAG rats showed the greatest decrease in that time, whereas the BN subjects showed a less marked response (Fig. 5). Animals returned to the pre-manipulation time spent in this zone in sessions 19 and 20.

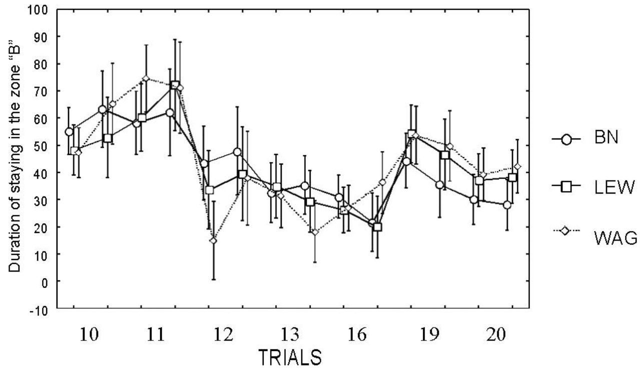

Fig. 5. Duration of time that rats spent in the control tunnel zone over recorded sessions. Each bar represents the three minute period of measurement within a given trial.

All subjects spent more time inside the experimental tunnels after introduction of novelty into the chamber. The BN rats' response was less apparent (Fig. 6). There were also significant differences between the strains in the course of habituation, with a clear habituation effect found in WAG rats and no signs of habituation in BN rats (the response of LEW rats was in between those two extremes).

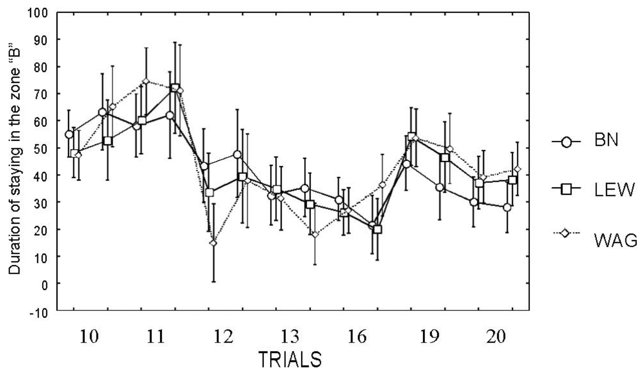

Fig. 5. Duration of time that rats spent in the control tunnel zone over recorded sessions. Each bar represents the three minute period of measurement within a given trial.

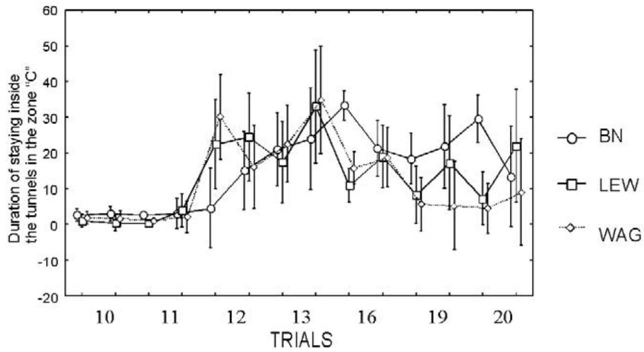

Fig. 6. Duration of time that rats spent inside the experimental tunnels over the recorded sessions. Each bar represents the three minute period of measurement within a given trial.

No effects were found on grooming, freezing, and stretching behavior.

EXPERIMENT II

The purpose of this experiment was to evaluate the influence of the experience of continuous environmental change on the response to novelty. Since continuous change creates a unique opportunity to experience various levels of complexity and intensity of stimulation, it seems reasonable to expect that this kind of experience modifies the response to a novel event.

Methods

SUBJECTS

WAG (WISTAR ALBINO GLAXO) and LEWIS rats were obtained from the Medical Research Center of the Polish Academy of Sciences, in Warsaw. They arrived in our laboratory at the age of seven weeks. Forty subjects (ten females and males of each strain), about 90 days of age at the onset of the experiment, were used. They were handled mildly daily by being tickled carried by human hand. Rats were housed in transparent plastic (polycarbonate) cages (610L, 415W, 215H mm, floor area 2530 cm^2), five subjects per cage. The housing conditions fulfilled the criteria set by the local and European Ethics Commission.

APPARATUS

The device described in Experiment I section was used.

PROCEDURE

The procedure used in this experiment was very similar to the one used in Experiment I. The main difference was that in case of the experimental group of rats, in section "C" of the experimental chamber, a persistent change of tunnel arrangement was introduced in the course of the experiment's habituation phase. Details of the procedure are shown in Figs 7 and 8.

Sessions 10 through 13 were video recorded. As in Experiment I, all six-minute sessions were divided into two three-minute intervals for the analysis. A four-factor MANOVA, involving $2_{(sex)} \times 2_{(strain)} \times 2_{(manipulation)} \times 8_{(3\text{-min interval})}$ was performed.

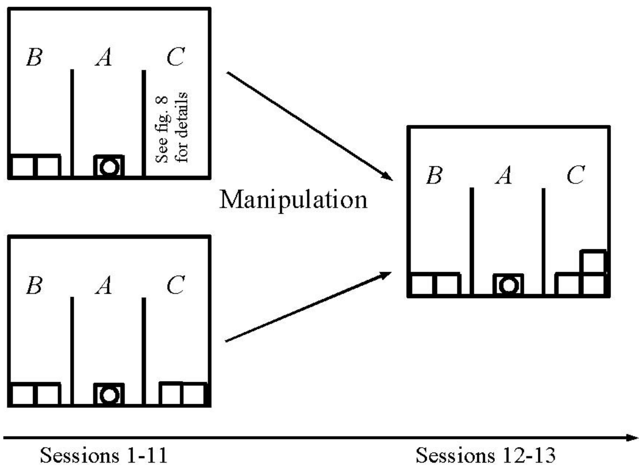

Fig. 7. Experimental conditions in Experiment II. Sessions 10 through 13 were video tapped. All the tunnels showed in the chamber could be entered by rats.

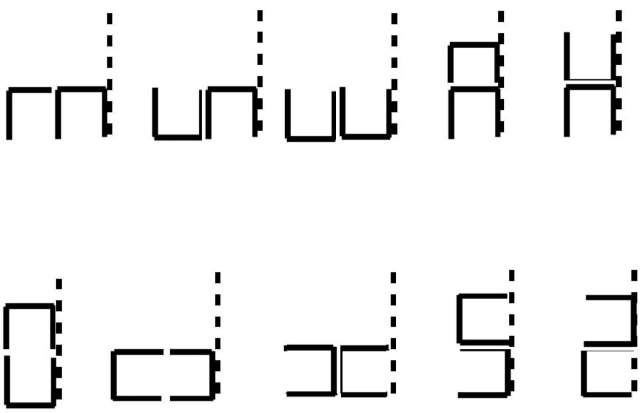

Fig. 8. Tunnel's arrangements in zone "C", applied in the habituation phase of Experiment II. Dotted line stands for the right wall of the chamber.

Results

There was an interaction between the interval and manipulation found for the time spent in the start zone (zone "A") of the experimental chamber; ($F_{7,\ 196}$=3.58, P<0.001). This result is shown in Fig. 9. The rats from the control group, who were exposed to the stable environment conditions (SE group) decreased the time spent in the start zone more significantly then their counterparts from the experimental, persistent novelty (PN), group. It should be noted, however, that during the habituation phase of the experiment, the SE rats were allocating more time to the start zone than the PN rats. Moreover, as is clearly seen in Fig. 9, the SE rats showed a return to the previous level of time allocation in session 12, while PN rats continued to spend time outside this zone.

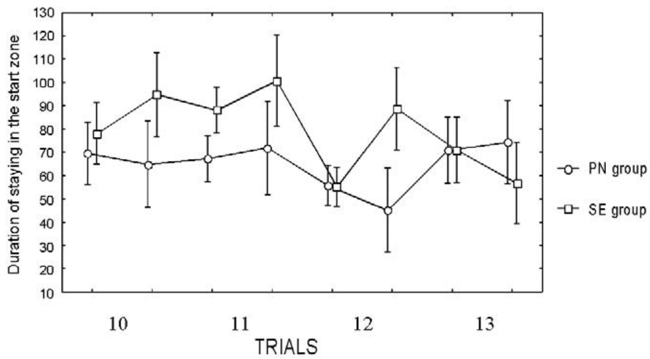

Fig. 9. Duration of time that rats spent in the start zone over recorded sessions

All the animals responded to the manipulation with decreased time allocated to zone "B" of the chamber, ($F_{7,\ 189}$=6.79, P<0.001). This result is shown in Fig. 10.

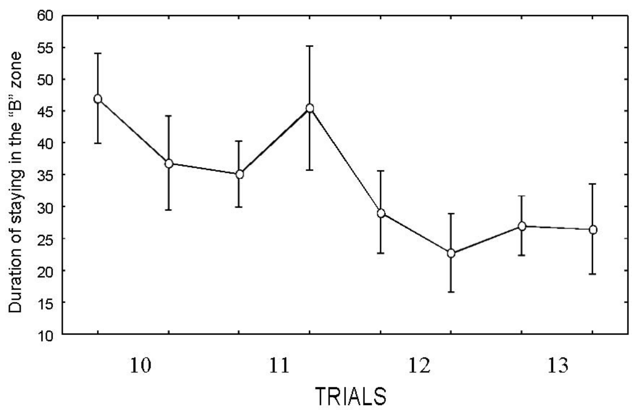

Fig 10. Duration of time that rats spent in the control tunnel zone over recorded sessions

All rats increased the durations of the zone "C" inspection in response to the manipulation. Interestingly though, SE subjects demonstrated withdrawal from this zone in the second half of the twelfth session as compared with the PN subjects. This resulted in an interaction between interval and manipulation factors: ($F_{7, 196}$=3.93, P<0.001). This finding is shown in Fig. 11.

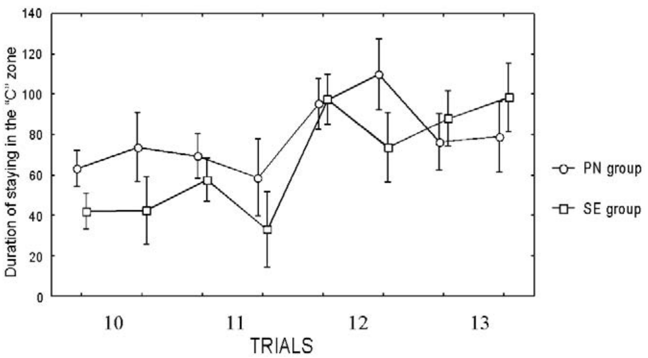

Fig. 11. Duration of time that rats spent in the experimental tunnel zone over recorded sessions

The analysis of rat contacts with the tunnels also showed an interaction (interval by manipulation) effect ($F_{7,196}$=3.54, P<0.002). SE subjects interacted with objects more than their counterparts in the course of thirteenth session. The immediate response during session 12, was very similar in both groups. Figure 12 shows the details of these findings.

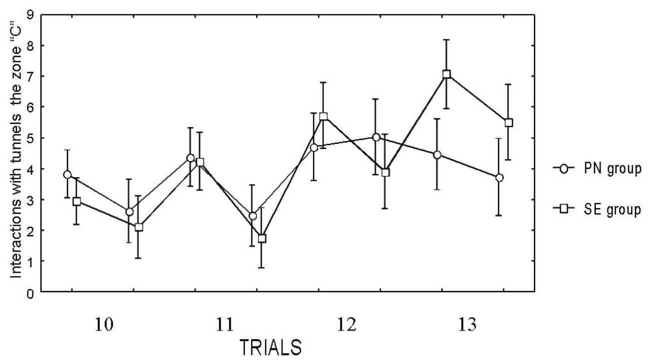

Fig. 12. Interactions with tunnels in the experimental tunnel zone over the recorded session

DISCUSSION

Pisula (2003) showed that under low- or non-stressful conditions rats demonstrate a positive, directed response toward the source of novel stimulation. This finding contradicts a traditional view that emphasized a neophobia-like phenomenon, namely stress and emotional responses, which are observed during behavioral activities described as SSDRs (Fanselow and De Oca 1998).

The present experiment was designed to eliminate the emotional response to novelty, while allowing us to investigate other sources of responses to novelty. Three strains of rats were systematically handled and provided with an opportunity to adapt to the experimental cage during several days of exposure. The subjects showed no signs of increased emotional response, such as freezing, grooming, or burying. This finding indicates that the procedure was not stressful, and provides additional support for the view that the procedure involving repetitive placement in the experimental cage under non-stressful conditions is an effective method of reducing novelty-related anxiety in rats.

The results indicate that emotional responses associated with a novel event have been effectively eliminated in this study. Therefore, the differences in behavior obtained after introduction of novelty may be attributed to individual characteristics associated with novelty-related behavior.

Coping with novelty is a major adaptive and psychological issue (Chown 1999, Heckhausen 2000). The way animals cope with a novel situation may be discussed within three frameworks: space preference, form of activity, and habituation.

Space usage

Some interesting conclusions may be drawn from the assumption that the time spent in particular zones of the cage is a measure of space preference. The tunnel zones (both control and experimental) were equally attractive to rats. The animals demonstrated a slightly stronger preference to stay in the start zone (80 to 90 seconds per measurement period), than in the tunnel zones (40 to 60 seconds). One could have expected that rats should show preference for the zone with two tunnels, as compared to the zone with one tunnel, since we know that rats usually prefer more complex environments. There were basically three stages of space usage adopted by rats in this study. The first one was established during the habituation phase, and it was observed in the 10th and 11th recorded session. This observation may be interpreted as an expression of the rats preferences for specific conditions, such as light intensity and environmental complexity. Then the novelty related behavior emerged, which was observed in the 12th, 13th, and to some extent, the 15th session. At the end of measurement, during the 19th and 20th session, space usage reflected space preference dependent on the properties of the stimulation generated by the rearranged chamber.

Habituation

Two distinct patterns of habituation were found in this study. The first was full habituation as in the case of duration of the time spent in the start zone. This is an important finding, as it proves that if the properties of the given area remain unchanged, the rat's behavior tends to recover from an initial disturbance to a level comparable to the earlier, pre-manipulation stage of the experiment.

The second pattern of habituation, related to the time spent by rats in both tunnel zones, may be called incomplete or partial habituation. What this means is that rats failed to restore the time budget allocated for these zones to the pre-manipulation level. It may be hypothesized that since the properties of the environment in the experimental tunnel zone, such as complexity and ecological attractiveness (more tunnels/shelters) have changed, the preference for given properties unrelated to novelty may play the main part in shaping behavior. Interestingly enough, contrary to other test situations (Pisula and Osiński 2000), gender was found to play a secondary role in generating the differences between individuals. As was clearly showed in earlier studies (Pisula et al. 1992, Renner 1987), experience significantly modifies exploratory activity in both quantitative and qualitative aspects. Since the main variable affecting rat behavior in this study was novelty, it seems crucial to test the role of rat's experience with novelty. This was the purpose of the Experiment II.

The immediate response to novelty of rats from the PN and SE groups was very similar. All these rats allocated more time to zone "C" after experimental manipulation. However, rats adapted to stable conditions in this zone (SE group) responded with a significantly more investigation of the tunnels than their PN counterparts. The experience of frequent changes in low-stress conditions may be analyzed in terms of environmental enrichment. The classic studies (Renner 1987, Renner and Rosenzweig 1986) show that rats exposed to enriched housing conditions manifest more sophisticated and elaborate interactions with investigated objects. In the present study, rats exposed to permanent change in the zone "C" spent less time interacting with tunnels then SE rats. The interpretation of these results is bidirectional. First, we may conclude that a constantly changing environment is not in fact an enriched environment: unstable does not necessarily mean enriched. The other option to be explored in the future is the potential specific effect of novelty on rat behavior. Subjects adapted to permanent change in zone "C" of the experimental chamber found this zone unattractive in the 13th session, since it has not changed from session twelve.

CONCLUSIONS

The results of this study confirm the findings obtained in previous experiments with Roman High- and Low avoidance rats (Pisula 2003, Pisula and Osiński 2000), as well as some classic studies. Rats in the familiarized conditions clearly showed a positive response toward the source of the stimulus field change. They also reorganized their behavior in terms of behavioral content. They systematically presented a shift from a somewhat schematic and routinely performed locomotor inspection toward more advanced, goal-directed investigation. Though the concept of a goal was not sufficiently elaborated, it is clear that exploring a novel object or area is a cognitive process, at least in higher vertebrates. Experiments exploiting manipulations with novelty of various degrees of intensity and quality may offer important insights into animal cognition.

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