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Behavioural Processes 68 (2005) 135–144
BEHAVIOURAL PROCESSES
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Anxiety levels and wild running susceptibility in rats: assessment with elevated plus maze test and predator odor exposure

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Received 28 October 2003; received in revised form 26 July 2004; accepted 13 December 2004

Abstract

It is reported in the literature that nearly 20% of rats are susceptible to displays of wild running (WR) behavior when submitted to high intensity acoustic stimulation. Some characteristics of WR suggest that it can be viewed as a panic-like reaction. This work aimed to test whether WR-sensitive rats show higher levels of anxiety in elevated-plus-maze (EPM) and predator–odor exposure paradigms in comparison with WR-resistant ones. Male adult Wistar rats were submitted to two trials of acoustic stimulation (104 dB, 60 s) in order to assess WR susceptibility. Seven WR-sensitive and 15 WR-resistant rats were evaluated by the EPM test. Other 13 WR-sensitive and 18 WR-resistant animals were submitted to the predator–odor exposure test which consisted of a 10 min-session of free exploration in a specific apparatus containing two odoriferous stimuli: cotton swab imbedded with snake cloacal gland secretion or with iguana feces (control). WR-sensitive rats presented a significantly higher closed-to open-arm-entry ratio in the EPM test. All rats responded with anxiety-like behaviors to the predator odor exposure, although the WR-sensitive ones showed a marked behavioral inhibition regardless of the odor condition. We conclude that WR-sensitive rats present elevated levels of anxiety manifested by means of passive behavioral strategies.

Keywords: Anxiety; Elevated-plus maze; Panic; Predator odor; Rat; Wild running

1. Introduction

The study of defensive behavior of animals achieved great importance in scientific research, since it was considered useful to unfold the biological basis of emotions

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doi:10.1016/j.beproc.2004.12.003

(Ledoux, 1996). In mammals, it has been described that such behaviors are organized in a hierarchical structure that adjust the animal's reaction to the degree of danger in each situation (Hendrie et al., 1996). Based on that observation and supported by consistent experimental data, eminent authors propose that the initial steps of the hierarchical structure, which consist of behaviors related to risk assessment and coordinated escapes, are accompanied by anxiety (Graeff, 1994; see a discussion in Andreatini et al., 2001). Consequently, the final steps are motivated by the panic state, which is observed in animals by means of dramatic reactions to avoid hazards and, in rats is manifested by typical defensive fighting and vigorous flight (Blanchard et al., 1984; Hebert et al., 1999).

There has been additional interest in research concerning panic reactions because panic disorder is recognized as a very debilitating disease that affects 2–4% of the human population (Ballenger et al., 1998). In this connection, many animal models of panic have been developed based on different methodologies, such as electrical (Brandão et al., 1994) or chemical (Schenberg et al., 2001) stimulation of brain sites, exposure to elevated mazes (Teixeira et al., 2000), lactate infusions (Furlan and Hoshino, 2001) and social grouping after REM sleep deprivation (Sandrin and Hoshino, 1999). Although each specific experimental model is not essentially the panic disorder manifestation itself, the models have supplied a means to investigate many important questions about this anxiety disorder.

Audiogenic seizure paradigm is one animal model of generalized convulsion (Ross and Coleman, 2000) whose neural base involves a large number of coincident brain structures associated with panic reactions (Beckett et al., 1997; Lamprea et al., 2002; N'Guemo and Faingold, 1998; Garcia-Cairasco et al., 1993). Curiously, the tonic–clonic fit observed in this paradigm usually starts as a locomotor pattern called wild-running (WR) behavior, which closely resembles panic flight. Prior studies from our laboratory showed a direct correlation between the susceptibility to presenting defensive fighting induced by REM sleep deprivation and WR manifestation (de Paula and Hoshino, 2002). In addition, strychnine administered at a sub-convulsive dose exerts facilitatory action upon both defensive fights and WR (de Paula and Hoshino, 2004). Finally, WR can be reduced by anti-panic procedures such as dorsal periaqueductal gray lesion and imipramine treatment (de Paula and Hoshino, 2003). These findings suggest that WR may be considered a panic reaction, but additional evidence must be pursued.

In normal rat populations, nearly 20% are susceptible to displays of WR when submitted to high-intensity acoustic stimulation (Romanova et al., 1993). Also, it is already known that among colonies of rats, some of them show higher levels of anxiety (Ramos et al., 2002). So, given the possibility that WR is a panic reaction, it is reasonable to suppose that WR-sensitive rats could be more anxious than others. Aiming to test this hypothesis, the present work assessed the anxiety levels of rats with and without WR susceptibility by means of conventional elevated-plus-maze test and predator odor exposure.

2. Materials and methods

2.1. Subjects

Adult male Wistar albino rats, weighing 250–350 g at the beginning of the experiments, were used. They were bred at the UNESP Central Animal House in Botucatu (SP/Brazil) and maintained for at least 1 week before starting experiments in our laboratory conditions. During this period, they were housed in groups of five animals in conventional polypropylene cages (40 cm × 32 cm × 16 cm) containing wood shavings on the floor and having potable water and food (Labine chow) accessible ad libitum. Cages were kept at a temperature of 25 ± 3 °C in a light/dark-cycle controlled room and were regularly cleaned every 2 days. All recommendations for ethical usage of animals stated by the Colégio Brasileiro de Experimentação Animal (COBEA) were followed.

2.2. Determination of WR susceptibility

Wild-running susceptibility was assessed by means of the high-intensity acoustic stimulation trial routinely conducted in our laboratory. The trial started by placing the rat in a wire mesh cage (33 cm × 25 cm × 19 cm) located inside a sound-proof chamber (40 cm × 33 cm × 29 cm) containing a ringing bell, an incandescent lamp bulb (60 W), and a glass window through which complete visualization of the

rat's behavior is possible. Fifteen seconds after the rat placement, the ringing bell was turned on, producing an acoustic stimulation of 104 dB applied continuously for up to 60 s, or until the rat emitted one clearly identifiable episode of WR. WR was operationally defined as a behavioral pattern that usually started with a sudden rotation of the body that was immediately followed by a high-speed circular running fit. Frequently, the running was so violent that it became an explosive flight marked by galloping, jumping and collisions against the walls of the cage. The rat was observed in real time, and was promptly considered sensitive to WR upon having displayed the complete pattern. In such a case, the stimulation was interrupted to avoid the progression to a convulsion, which was held unnecessary in the present study. It is important to report that even short episodes of WR, lasting 2–4 s and consisting only of some running laps around the cage served as positive indicator of WR susceptibility. This happened because commonly the short running repeats along with stimulation evolving to dramatic flights and convulsions (personal observations). The WR-resistant rats behave very differently in the trial, showing no signs of locomotor agitation or vigorous attempts to escape from the cage.

In the present work, 121 rats were tested, and 20 (16.5%) were considered WR-sensitive (WR-s) animals. From the remaining 101, only 33 WR-resistant rats (WR-r) were randomly selected to be compared with the WR-s rats in the behavioral tests described below. One week after being submitted to the behavioral tests, all 53 rats had their WR susceptibility confirmed in a second acoustic stimulation trial.

2.3. Behavioral tests

2.3.1. Elevated plus maze test

Seven WR-sensitive and 15 WR-resistant rats were submitted to the elevated plus maze (EPM) test in a standard wooden apparatus consisting of two opposite open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 40 cm) perpendicularly positioned that emanate from a central square (10 cm × 10 cm) elevated 50 cm from the floor. The open arms were surrounded by a 1 cm translucent Plexiglas ledge. The test was composed of a single free exploration session lasting 300 s, conducted during the light period of the day in a silent room in the laboratory. Before and after each session, the apparatus was cleaned with ethanol 10%. All the experiments were recorded with a video camera and, in a posterior behavioral transcription, a trained observer blind to the conditions counted the time spent in the arms and the number of entries. Data converted to a continuous scale, such as percentages of time and closed-to-open-arm-entry ratio, were analyzed by Student *t*-test for independent samples of unequal size. The total number of entries was analyzed by Mann–Whitney *U* test. Both tests were conducted using a specific software (Statistitica/Stasoft) with significance level set at 5%.

2.3.2. Predator odor exposure test

The predator odor exposure test was conducted in an apparatus adapted from a conventional polypropylene cage (40 cm × 32 cm × 16 cm), as depicted in Fig. 1. Adaptations consisted of the replacement of one opaque longitudinal wall (40 cm × 16 cm) with a transparent one that allowed complete visualization of the inner space. Also, a 10 cm-diameter opening in one of the transversal walls was made in order to connect an entrance module, adapted from a glass pot with the same diameter. The odor source (a cotton swab impregnated with odoriferous solutions) was attached to the cage corner on the right side of the entrance opening. In the middle of the cage, an opaque curtain (32 cm × 16 cm) made of black plastic film was installed transversely, so that the inner space of the cage was divided into two compartments: one with and the other without the odor source. Thus, the complete apparatus consisted of the entrance module plus the

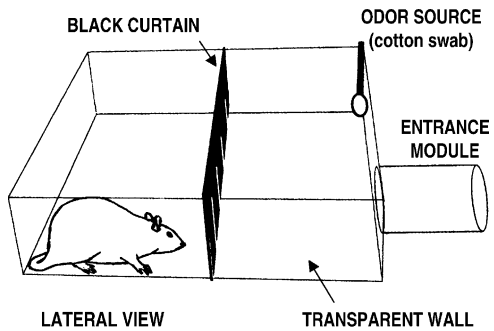

Fig. 1. Schematic representation of the apparatus used in the predator odor exposure test. It was made by adapting one conventional polypropylene cage (40 cm × 32 cm × 16 cm) with the addition of the entrance module indicated in figure. The cotton swab represents the odor source used in the tests.

two cage compartments. Before starting the behavioral test, the apparatus was wrapped with transparent cling film to provide odor insulation.

Thirty-one subjects were assigned to one of four groups, two of which were exposed to the predator odor and two serving as controls. The predator odor condition was produced with the usage of snake odor, that was released from a formaldehyde solution (10%) containing the cloacal scent gland secretion of one adult anaconda (*Eunectes murinus*). This solution was produced in the Bauru Zoological Park (SP/Brazil) when one just-dead snake was immersed in the formaldehyde solution and released the brown-colored secretion into it. Samples from this liquid, that no longer was used to fixate the snake, were yielded by the Zoological Park to be tested as an odoriferous solution because it smells very similar to the snake's cage. Submitted to this condition was a group composed of nine WR-r rats and another containing seven WR-s animals. The control condition (applied to the other 9 WR-resistant and to 6 WR-sensitive rats) was produced with an equivalent repugnant odor, as the snake odor smells very pungent even to the experimenters. Thus, iguana (*Iguana iguana*) feces, also collected at the Bauru Zoo Park and dissolved in formaldehyde solution, were used because, although similarly pungent, they are derived from a herbivorous reptile. Odoriferous solutions, that had been kept refrigerated at 8 °C, were warmed up to room temperature at the time of the tests. Tests were conducted between 10:00 and 13:00 h in a quiet laboratory room separate from the animal colony but with similar environmental conditions. Procedures started by placing the rat into the entrance module that was immediately connected to the apparatus cage. Next, a 600 s session of free behavioral recording using a video camera was conducted. In order to avoid cross contamination of the odors, one kind of odor was tested as a treatment per day, and the apparatus cleaned with ethanol between sessions.

The many specific behaviors recognized on the tape recordings were transcribed by a trained observer blind to treatments and groups, using a specific software (Etholog 2.25; Ottoni, 1996). However, for statistical purposes, some behavioral items were grouped into generic units in order to represent more comparable parameters in the predator exposure test. Thus, the behavioral units used as variables in the present study were the following: (a) *olfactory exploration*, which consisted of all types of sniffing behavior that rat emitted to air, entrance module, separating curtain, walls and floor of the cage, except those directed to the odor source; (b) *risk assessment behaviors*, composed of stretched approach and sniffing specifically the odor source; (c) *non-defensive behaviors*, including grooming, walking, and sleeping activities; (d) *body immobility*, characterized by indistinctive stationary position; and (e) *freezing*, identified by the typical posture of alert immobility associated with exophthalmous, hyperventilation and intense vibrissa movements. Besides such units that were recorded as states due to their measurable duration, other behavioral units, transcribed as events and recorded in terms of frequencies, were also used as comparative parameters between the groups. They were: (a) *staring at the odor source*; (b) *head-out behavior*, when the rat hidden in the curtain stretched the head and neck beyond it; (c) *wall rearing*; and (d) *running*. In addition, the spatial distribution of rats throughout the experimental apparatus as well as fecal boli produced during the test were computed.

Duration (converted to percentage of time) and behavioral frequencies were analyzed by means of two-way ANOVA test (odor exposure and WR-susceptibility factors). Such analysis was made using a specific software (Statistica/Statsoft), with the significance level set at 5%.

3. Results

3.1. Elevated plus maze test

Means (±S.E.M.) of the parameters evaluated in the EPM are shown in Table 1. The percentage of time spent in the arms and in the central square did not differ significantly between groups according to the statistical test applied. The closed-to-open-arm-entry ratio detected for the WR-resistant rats was 0.78 ± 0.18 (mean ± S.E.M.), while for the WR-sensitive animals it was 2.23 ± 0.54. These values showed a significant statistical difference ($t = 3.21$; df = 20; $P = 0.004$). The total number of entries into arms was similar between groups.

3.2. Predator odor exposure test

During the tests, most of the rats spent a large amount of time (around 80%) in activities such as

Table 1
Means (±S.E.M.) of the parameters recorded in the EPM test for WR-resistant and WR-sensitive rats

Parameters	Groups	
	WR-resistant rats ($n = 15$)	WR-sensitibility rats ($n = 7$)
Percentage of time spent in open arms	20.20 ± 4.85	15.85 ± 4.32
Percentage of time spent in closed arms	62.73 ± 5.56	71.28 ± 5.14
% of time spent in center	16.27 ± 3.97	12.80 ± 2.92
Closed-to-open-arm-entry ratio	0.78 ± 0.18	2.23 ± 0.54*
Total number of entries in any arm	12.87 ± 1.12	12.85 ± 2.57

* Indicates statistically significant difference ($P < 0.05$) between WR-resistant and -sensitive groups.

olfactory exploration, grooming, and body immobility. However, as is revealed in Table 2, percentages of time (expressed as mean ± S.E.M.) spent in these and other behaviors were consistently altered by the experimental conditions. Two-way ANOVA revealed that exposure to predator odor was accompanied by increased time spent in risk assessment activities [$F(1,27) = 15.00$; $P < 0.001$], olfactory exploration [$F(1,27) = 21.88$; $P < 0.001$] and freezing behavior [$F(1,27) = 7.00$; $P = 0.01$]. In the same way, this condition (predator odor) reduced significantly the percentage of time in body immobility [$F(1,27) = 11.36$; $P = 0.002$] and in non-defensive behaviors [$F(1,27) = 28.85$; $P < 0.001$]. The WR susceptibility factor had a significant effect on reduced risk assessment activities [$F(1,27) = 4.23$; $P = 0.04$] and on increased body immobility [$F(1,27) = 5.30$; $P = 0.03$] recorded in groups composed of WR-sensitive rats. Interaction effects were not statistically significant for any of the above mentioned parameters. Results of the behavioral units recorded as frequencies are shown in Table 3. Two-way ANOVA revealed that the odor condition factor significantly effected higher frequencies of staring at the odor source [$F(1,27) = 13.92$; $P < 0.001$], head-out behavior [$F(1,27) = 4.45$ $P = 0.04$] and wall rearing [$F(1,27) = 6.61$; $P = 0.01$] recorded in predator odor-exposed groups. The WR susceptibility factor produced significantly lower frequencies of head-out behavior [$F(1,27) = 5.02$; $P = 0.03$] and running [$F(1,27) = 8.17$; $P = 0.008$] recorded in WR-sensitive groups. No interaction effects were statistically significant for such parameters. Data concerning the temporal distribution of rats in each of the apparatus compartments, presented in Fig. 2, were analyzed by a 3-way ANOVA with comparisons between the conditions (type of odor and WR susceptibility) and within the compartments (i.e. time spent in entrance module versus compartment with odor source versus compart-

Table 2
Means ± S.E.M. of the behavioral parameters measured as percentage of time in WR-resistant and WR-sensitive groups submitted to snake (predator) and iguana feces (control) odors

Parameters	Odor conditions	WR susceptibility		ANOVA effects (significance)		
		Resistant	Sensitive	Odor	WR susceptibility	Odor × WR
Risk assessment behaviors	Predator	14.2 ± 2.7	8.5 ± 1.1	$P < 0.001$	$P = 0.04$	NS
	Control	5.0 ± 1.4	2.9 ± 0.6			
Olfactory exploration	Predator	60.7 ± 2.5	56.4 ± 4.3	$P < 0.001$	NS	NS
	Control	40.8 ± 4.0	41.4 ± 3.5			
Freezing behavior	Predator	5.8 ± 1.9	6.2 ± 2.3	$P = 0.01$	NS	NS
	Control	2.7 ± 0.9	2.0 ± 0.8			
Body immobility	Predator	3.7 ± 1.3	11.0 ± 2.6	$P = 0.002$	$P = 0.03$	NS
	Control	14.8 ± 4.0	23.9 ± 5.7			
Non-defensive behaviors	Predator	14.0 ± 2.6	15.9 ± 1.6	$P < 0.001$	NS	NS
	Control	35.4 ± 3.8	28.8 ± 3.4			

NS = non-significant "P" value ($P > 0.05$).

Table 3
Means ± S.E.M. of the behavioral parameters measured as frequencies in WR-resistant and WR-sensitive groups submitted to snake (predator) and iguana feces (control) odors

Parameters	Odor conditions	WR susceptibility		ANOVA effects (significance)		
		Resistant	Sensitive	Odor	WR susceptibility	Odor × WR
Staring at the odor source	Predator	8.33 ± 1.01	7.14 ± 1.65	$P < 0.001$	NS	NS
	Control	4.00 ± 0.57	3.16 ± 1.13			
Head out	Predator	6.44 ± 1.09	3.28 ± 1.17	$P = 0.04$	$P = 0.03$	NS
	Control	3.44 ± 1.43	1.66 ± 0.47			
Running	Predator	3.44 ± 0.68	1.28 ± 0.52	NS	$P = 0.008$	NS
	Control	2.00 ± 0.68	0.50 ± 0.34			
Wall rearing	Predator	24.0 ± 3.2	20.1 ± 1.6	$P = 0.01$	NS	NS
	Control	14.7 ± 3.5	12.8 ± 3.2			

NS = non-significant "*P*"-value ($P > 0.05$).

ment without odor source). Accordingly, there was a significant effect regarding the temporal distribution of rats throughout the compartments [$F(2,50) = 3.62$; $P = 0.03$] independent from the odor condition or WR susceptibility. It means that, in general, rats spent significantly less time inside the entrance module (21.2 ± 5.6%; mean ± S.E.M.) compared to the compartment without the odor source (43.1 ± 4.7%), where they spent the most time. An intermediate value (35.7 ± 4.0%) was recorded for the compartment *with* the odor sources. There was a marginally significant interaction effect involving such distribution and the odor conditions [$F(2,50) = 2.77$; $P = 0.07$] as the predator odor-exposed groups showed very short duration inside the entrance module (11.3 ± 2.7% of time) in contrast with the control odor condition (30.9 ± 10.1%). In addition, the WR-resistant rats exposed to the predator odor spent a large amount of time inside the compartment with the odor source: 52.2 ± 5.4%. This trend was not followed by the WR-sensitive rats (see Fig. 2), which probably contributed to the failure to reach the significance level for the interaction effect. Interaction between temporal compartment distribution and WR susceptibility was not statistically significant, as well as the interaction of the three factors.

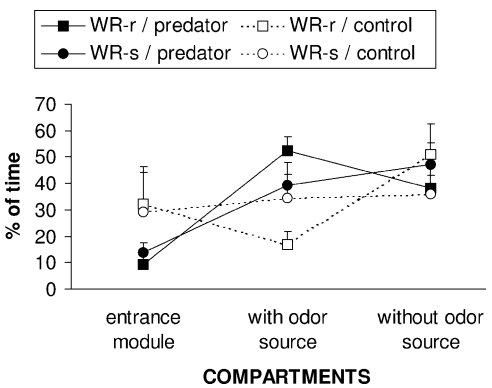

Fig. 2. Temporal distribution of rats in each of the apparatus compartments (see Fig. 1) expressed as mean ± S.E.M. of time percentage for each group. WR-r and WR-s represent wild running-resistant and -sensitive groups, respectively, exposed to predator odor (snake cloacal gland scent) or to the smell of iguana feces used as control.

The number of transitions between the compartments was 32.2 ± 8.4 and 35.2 ± 6.8 for groups submitted to control odor condition, and 71.0 ± 5.5 and 52.3 ± 5.2 for predator odor-exposed groups regarding WR-resistant and WR-sensitive rats, respectively. Two-way ANOVA revealed a significant effect of the odor condition [$F(1,25) = 13.46$; $P = 0.001$] with the predator odor-exposed groups presenting higher levels of locomotor activity. Interaction effects did not present statistical significance.

Counts of fecal boli found in the apparatus determined that WR-resistant and WR-sensitive iguana feces odor-exposed groups produced 1.8 ± 0.4 and 1.8 ± 0.8 (mean ± S.E.M.) during the 10 min session. Regarding predator odor-exposed groups, WR-resistant and WR-sensitive produced 4.0 ± 1.3 and 5.1 ± 1.6, respectively. Analysis showed significant effect of the odor exposure factor [$F(1,27) = 5.31$; $P = 0.02$] and no significant effect regarding WR susceptibility or the interaction between them.

4. Discussion

The results obtained in the experiments allow us to conclude that wild-running susceptibility in rats is accompanied by elevated levels of anxiety. However, such anxiety is expressed in WR-sensitive rats in a particular manner, showing features of passive strategy to deal with dangerous situations. This interpretation is important to understand the central idea here discussed, that is the apparent paroxystical pattern of some animals' behaviors in defensive contexts.

In the first experiment, the EPM test was used to compare anxiety levels of the two types of rats considered herein. The EPM test is a widely accepted animal model to access anxiety in rodents and is shown to have neurophysiological, pharmacological and behavioral validity (Pellow and File, 1986; Duncan et al., 1996). In the present work, WR-sensitive rats entered inside the closed arms twice as much as they moved forward the open arms. This profile was not observed in the resistant rats and can be considered an indicator of high levels of anxiety. The lack of other reports in the literature concerning behavioral profiles of WR-sensitive rats in the EPM test do not allow direct comparisons; but data derived from audiogenic seizure (AS)-susceptible rats and kindled animals can be useful. Although important differences exist between the three types of rats, such as genetic manipulation in AS-sensitive rats (Garcia-Cairasco et al., 1990) and chronic experimental procedures in kindled rats (Pinel and Rovner, 1978), they all share a propensity to manifest violent pre-convulsive running fits more often than other animals (Romanova et al., 1993; Kalynchuk, 2000). Garcia-Cairasco et al. (1998) found that AS-sensitive rats have a reduced percentage of time spent in open arms compared to resistant ones. This can be a clear indicator of a higher anxiety level, although the animals also showed overall activity reduction in the EPM test (including closed-arm parameters) as well as in the open field. This fact suggests the reduced exploratory activity as a possible confounding factor. In the present study, the overall reduced exploration was not observed in EPM since the total number of entries was equivalent among groups. This finding reinforces the hypothesis of the higher anxiety level. In addition, temporal-lobe electrically kindled rats also are less active in the open field and were shown to be very anxious in a variety of emotionality tests, including EPM (Kalynchuk et al., 1998). Therefore, it can be assumed that WR susceptibility in rats is frequently associated with higher levels of anxiety determined by the EPM test.

The predator–odor exposure test is indicated to be very useful, since it has a naturalistic appeal due to the simulation of a realistic situation in the rodent's life (Dielenberg and McGregor, 2001). In rats, stimulation with odoriferous substances derived from carnivorous mammalians classically induces risk-assessment related behaviors and reduces non-defensive activities such as resting, feeding and self-cleaning (Blanchard et al., 1998). This pattern is specifically produced by the signals from predator odor and is different from reactions provoked by neutral stimuli with repugnant characteristics, including formaldehyde (McGregor et al., 2002), that only activate anxiety-like responses after repeated exposures (see Sorg et al., 2001). Although there are no reports of rat responses to snake odor, it was observed that mice consistently react to such odor with risk-assessment related behaviors (Dell'omo and Alleva, 1994; Carere et al., 1999), in a very similar fashion with exposure to mammalian carnivore odors (Berton et al., 1998). In our interpretation, the snake odor used in the current work showed a recognizable anxiogenic effect in rats due to the following reasons: Firstly, all rats exposed to the snake odor reacted with increases in risk assessment, olfactory exploration, freezing and odor-source-directed behaviors, while non-defensive activities were consistently reduced in comparison with the exposure to iguana feces odor. Secondly, exposure to snake odor was accompanied by intense defecation that is a widely accepted indicator of anxiety and fear in rats (Denenberg, 1969; Plyusnina and Oskina, 1997). Finally, the time spent in a place with no escape alternatives, like the entrance module made of glass, tended to be reduced in the snake odor condition. Thus, the anxiogenic property of the snake odor was shown to be effective also in rats. Interestingly, the rats did not have prior contact with snakes and belonged to a strain bred in laboratories for a long time. Probably, the ancient and intense predation relationship with snakes has favored the rodents that developed a powerful and innate mechanism of snake detection (Bramley et al., 2000). Also, their nocturnal habit must have demanded that such a mechanism should function by means of olfactory signaling.

Assuming that the snake odor worked as an anxiogenic factor, what was the behavioral difference between the two sub-populations of rats divided on the basis of WR susceptibility? The lack of interaction effects in the statistical analyses reveals that they were not differently affected by the predator odor specifically. Instead, WR-sensitive rats presented less overall risk-assessment activity and more time spent in body immobility independently from the odor condition. This profile closely resembles the behavioral inhibition observed in rats submitted to the open field (Plyusnina and Oskina, 1997; Ramos et al., 2003), which leads to the idea that, as rats were not previously habituated to the apparatus, novelty could be the main factor that contributed to such results. Actually, this was somehow predictable and the reason we opted for not habituating the rats to the odor apparatus. The hypothetical less explorative behavior of the WR-sensitive rats (already reported for AS susceptible-rats by Garcia-Cairasco et al., 1998) could be confused with avoidance of predator odor exhibited by habituated rats. But, in a novel environment, all rats were forced to explore it, which generated a conflict between two opposite tendencies: exploration and avoidance. It was observed that the predator odor increased apparatus exploration and odor-source investigation (raising risk assessment activities) in all rats, suggesting that in the test conditions predator odor might have activated some kind of arousal or vigilance mechanism. This could be responsible for raising overall exploration in the novel environment and for suppressing, at least in a first moment, the avoidance response. Curiously, the WR-s rats were less exploratory independent from the odor, indicating that odor avoidance may not be posited as the principal factor leading to the behavioral inhibition seen in these rats. Therefore, it is possible to state that the novelty of the context might have selectively influenced the behavior of WR-sensitive rats with the emphasis that, despite this, they also were shown to be affected by the anxiogenic property of snake odor. Analyzing this result together with the EPM findings, it becomes valid to ask: Why do WR-sensitive rats react with fewer exploratory activities compared to the WR-resistant ones in anxiogenic situations that characterized the behavioral tests? In our view, it could be explained by a possible association between wild running susceptibility and passive strategies to cope with danger.

Different styles of coping with aversive stimuli have been increasingly documented, and two of them, called active and passive strategies, are reported to have distinct neural function as well as physiological and behavioral outputs (Roozendaal et al., 1997). Of interest is the passive strategy, as it seem to be the coping style adopted by WR-sensitive rats in the current study. The passive strategy consists of reduction in general activity and a reactive pattern predominantly commanded by external stimuli that seem to be especially adaptive in unpredictable situations (Benus et al., 1991). These features make the passive strategy more difficult to regard as an anxiety response in exploration-based tests, as it was in the predator odor exposure test. But the passive strategy is frequently accompanied by typical anxiety symptoms such as high adrenocortical activation and autonomic alterations (Bohus et al., 1987). In WR-sensitive rats, this was not directly assessed by the present work, but the marked reduction in explorative behaviors can be considered a putative evidence of passive defense style.

One may think that the association between the wild running propensity, which is also correlated with defensive fighting (de Paula and Hoshino, 2002), and the passive defensive style is improbable, since fight-or-flight reactions are considered active forms of defensive behavior (Roozendaal et al., 1997). However, the panic reactions that comprise the fight-or-flight behaviors considered herein are stereotyped responses manifested when safe routes to escape are not available (Blanchard et al., 1993). Undoubtedly, this represents one advantageous hazard-avoidance option for animals whose reduced exploration (active defense) did not provide anticipatory alternatives. Curiously, the defensive pattern comprised, at the same time, of low activity and paroxystic fight-or-flight reactions are independently described for the rats susceptible to wild running. Kindled rats, after being motionless in open field trials, can violently attack the experimenter who tries to catch them (Kalynchuk et al., 1999). Significantly increased startle response is observed in rats genetically selected for presenting pronounced freezing behavior (Popova et al., 2000). Behavioral descriptions (Garcia-Cairasco et al., 1994) and personal observations (unpublished) of rats submitted to audiogenic acoustic stimulation corroborate that the sensitive rats frequently stay immobile before precipitating the wild running. In contrast, the WR-resistant rats usually show

exploratory or grooming behavior during the acoustic stimulus presentation. Such data support the idea that some rats defend themselves through a paroxystic pattern that associates passive strategies with panic reactions. In this connection, the studies of genetically developed rats presenting different levels of amygdala excitability ("slow" and "fast" kindling rats) conducted by McIntyre et al. (1999) are very illustrative. It is reported that "slow" rats are more prone to adopt a passive defensive style compared to "fast" rats when submitted to a variety of stressors, with the only exception being exposure to a predator, when the "fast" rats display marked immobility (McIntyre et al., 1999). Like the cited work, we found a passive behavioral strategy in a kind of rat for which active responses would be expected due to WR susceptibility. This demonstrates the potential complexity of the relationships between defensive style and neural functioning and also the unpredictability of reactions to different behavioral tests.

The original purpose of the present work was to attribute an anxiety level to the WR-sensitive rats, as their propensity to manifest wild running can be viewed as a panic susceptibility. So, the hypothesis was that propensity to panic would be related to ultimate anxiety levels. However, the defensive style of animals rendered analysis of our results more complex, since some signs of anxiety appeared to be obscured by the reduced exploration of WR-sensitive rats. Thus, the best conclusion is that wild-running susceptibility is accompanied by increases in anxiety; and the passive strategy is one important factor that must be taken into account for the comprehension of this type of anxiety. This statement may be useful to understand the paroxystic feature of some animals' defensive behavior and to propose that opportunistic oscillations between active and passive defensive strategies may be possible.

Acknowledgements

We thank Luis Pires and Gerson Rodrigues from the Bauru Municipal Zoological Park for providing biological material and for collaborating with information on the cloacal scent gland of snakes. Financial support for conducting the present work was provided by CAPES/Brazil.

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