Behavioural Brain Research 208 (2010) 282–285

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Together they stand: A life-threatening event reduces individual behavioral variability in groups of voles

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ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form
26 November 2009
Accepted 30 November 2009
Available online 3 December 2009

Keywords:
Predation risk
Defensive behavior
Stress response
Stress in groups
Risk assessment
Predator–prey interactions

ABSTRACT

In the present study, voles were exposed to owl attack as a group, with their cage mates and in their familiar cages. The anxiety level of each vole was assessed using two parameters: time spent in the open arm of an elevated plus maze; and time spent away from the walls of an open field. Each parameter was measured 24 h before and after the group exposure to the owls. We found that the large individual differences in the voles' behavior measured before exposure to the owls were significantly reduced following exposure. In other words, after exposure all individual voles began to behave the same both in the elevated plus maze and in the open field. This response, as measured 24 h after the exposure to the owls, differs from past studies, where individual voles diverge in their immediate response in order to confuse the attacking owl. We suggest that the present finding on reduction in individual differences is a group effect reminiscent of the social response seen in humans following a disaster, when a uniform behavioral code dominates and trims down behavioral variability.

Rodents comprise the main component of barn owl (*Tyto alba*) diet, and previous studies have shown that they are highly sensitive to owls, responding both behaviorally (freezing or fleeing) and physiologically (increase in blood corticosteroids) even to just an owl call [1–5]. Specifically, individual voles under owl threat diverge in response: some freeze, others flee, and others alternate between freezing and fleeing, so that the owl can not predict the response of a specific individual vole [1–5]. However, while past studies focused on the immediate individual response to the owls, here we studied the behavior of groups of voles 24 h after the exposure to the owls, compared to the behavior of the same groups 24 h before the attack. We assess the effect of extreme stress exerted by live barn owl attack on the behavior of groups of caged social voles (*Microtus socialis*). These rodents are social burrow dwellers, weighting 37–50 g and 11 cm in length plus a 2-cm tail. They were selected for this study as a particular prey of owls, comprising 40–70% (sometimes over 90%) of the diet of barn owls and tawny owls (*Strix aluco*) [6–9]. Eighteen voles were obtained from breeding colonies at the I. Meier Segals Garden for Zoological Research at Tel-Aviv University. They were kept in groups of 4–7 in metal cages (60 cm × 30 cm × 20 cm) with a wire-mesh roof. Before testing, voles were marked individually by shaving a specific part of their fur, and then acclimated for two weeks in their cages inside a quiet air-conditioned room (24 °C) with 10/14 hrs light/dark cycles. Voles were daily fed *ad-libitum* with standard rodent pellets, sunflower seeds and fresh vegetables.

After two weeks, each individual vole underwent a pre-exposure test in an open field [10] and elevated plus maze [11]. Each vole was first tested for 15 min in the open field (2 m × 2 m arena with 50 cm plexiglas walls), and one hour later the same vole was tested for 5 min in the plus maze (a black-painted 70 cm × 70 cm aluminum cross placed horizontally 50 cm above the ground, with the sides of two arms closed by 20-cm high aluminum walls, and the other two open arms bordered with 5 mm low wall). The plus maze is a common test for anxiety: the greater the time spent in the closed arms during the 5 min test in the plus maze, the higher the anxiety level [11]. Similarly, the greater the amount of time spent in the open-field center, the lower the anxiety level [12,13]. All testing started at dusk, a peak activity time in the nocturnal social voles [6], and terminated before midnight. Testing took place in a quiet room illuminated by a dim light to provide the voles with a relatively non-aversive environment. An infrared light source (Tracksys, IR LED Illuminator; UK, with 830 nm filters that emits light not visible to rodents) was used to illuminate the apparatus for bright video recording by a video camera placed above the center of each apparatus.

The day after testing in the open field and plus maze, just before dusk, voles were exposed as a group to owl attacks. For this, the

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doi:10.1016/j.bbr.2009.11.045

voles' home-cages were transported to the center of a barn owl aviary (6 m × 6 m × 4 m), in which the owls could fly freely. The owls had been 1-day food-deprived prior to the test day. The owls' food (dead mice or chicks) was then placed on the wire-mesh roof of the voles' cage. Thus, when the owls swooped down on the vole cage to feed, they threatened the voles but could not reach them through the wire-mesh of the cage roof. The next morning, after spending overnight in the owls' aviary, the voles' cage was returned to the quiet acclimation room, and at dusk the same day, each vole underwent the post-exposure test of 15 min in the open field and one hour later, 5 min in the plus maze. The voles ($n = 10$) were thus exposed to owls in the same social groups in which they had lived prior to testing, but were individually tested in the open field and plus maze before and after the exposure to the owls. As control we used a second group of voles ($n = 8$) that underwent exactly the same testing procedures at the same time, but with their cage being placed in an empty aviary rather than in the owls' aviary. In other words, the controls underwent the same grouping and transfer procedures, and even owls' food was placed on their cages, but they were not exposed to owls that attack their group cage.

Behavior of voles in the open field was analyzed by means of 'Ethovision' software (by Noldus Information Technologies, NL), which tracks the progression of the vole in the arena, providing five times per second the time and the location of the center of the vole's image against the background of the brighter arena floor. From Ethovision, we obtained the *distance moved* (m), which was the cumulative distance traveled by a vole during a 15 min trial; *velocity* (m/s), which was the mean speed of traveling in the open field; and *center duration* (s), time spent in the center of the open field, at least 20 cm away from the arena walls. Behavior of voles in the plus maze was scored during playback of the video files, as follows: *open-arm time*, which was the cumulative time spent in the open arms; and percentage of open-arm entries, which was the number of open-arm entries divided by total arm entries. Arm exit (either closed or open) was scored whenever the rodent stepped out of an arm with at least two legs, while arm entry (either closed or open) was scored whenever the rodent stepped into an arm with all four legs.

Table 1 presents the parameters of vole activity before and after the overnight exposure in the owl cage (experimental group), compared to the same parameters in the control voles. As shown, during the post-exposure period there was a decrease in the time spent both at the center of the open field and in the open arm of the elevated plus maze (Table 1). While this was the average effect, changes at the individual level were more complex. In Figure 1a, individual voles are depicted along the X-axis (○), ranked from low to high according to the time spent in the open plus maze arm during the pre-exposure test. This ranking trivially displays an inclined trend (solid line), reflecting that some voles were more anxious (spent less time in the open) while others were less anxious (spent more time in the open). The respective post-exposure scores for each individual vole are depicted at the same rank (■). As shown, individuals showing lower ranking during the pre-exposure test went up in the post-exposure test, while voles with high ranking during the pre-exposure test went down in the post-exposure test. The trend in the post-exposure group (dashed line) is revealed as horizontal compared to the inclined trend of the pre-exposure group. Such change between pre- and post-exposure did not appear in the control group. Similarly, Figure 1b provides the data for time spent in the center of the open field, under the same layout of Figure 1a, and illustrates the same changes that were noted in the elevated plus maze. Altogether, changes in voles exposed to the owls were similar for both time spent in the open plus maze arm and time spent in the center of the open field, reflecting a post-exposure convergence of the

Table 1
Behavioral parameter in the open field and the elevated plus maze, and the results for the ANOVA comparisons of between-group effect (voles exposed to owls vs. control voles), within-group effect (testing before and after exposure to the owls), and the interaction between the between-group and within-group effects. Significant changes are depicted in boldface characters.

	Group exposed to owls		Control group		Between group effect ($F_{1,16}$; p)	Within group effect ($F_{1,16}$; p)	Interaction
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure			
Open field							
Distance moved (m)	78.24 ± 10.05	100.1 ± 8.7	62.95 ± 14.03	80.49 ± 12.61	2.09; 0.16	4.94; **0.04**	0.05; 0.8
Velocity (m/s)	0.09 ± 0.01	0.11 ± 0.01	0.07 ± 0.02	0.09 ± 0.01	2.02; 0.17	5.11; **0.03**	0.04; 0.83
Center duration	35.30 ± 6.32	23.48 ± 4.4	18.18 ± 6.07	13.6 ± 3.99	6.81; **0.019**	2.66; 0.12	0.52; 0.48
Plus maze							
Open arm (s)	55.64 ± 13.54	40.45 ± 4.30	18.63 ± 5.48	23.63 ± 5.12	9.7; **<0.007**	0.47; 0.5	1.81; 0.19
Total number of arm entries	27.90 ± 3.57	25.10 ± 1.92	13.00 ± 1.67	17 ± 1.58	20.42; **<0.001**	0.2; 0.66	6.55; **0.02**
% of open-arm entries	0.35 ± 0.04	0.27 ± 0.02	0.22 ± 0.04	0.28 ± 0.03	2.01; 0.18	0.12; 0.74	7.31; 0.02