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RAPID COMMUNICATION

Responders and Nonresponders to Cat Odor Do Not Differ in Other Tests of Anxiety

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HOGG, S. AND S. E. FILE. *Responders and nonresponders to cat odor do not differ in other tests of anxiety.* PHARMACOL BIOCHEM BEHAV 49(1) 219–222, 1994. – Laboratory-bred rats can be divided into those showing clear innate behavioral responses to the odor of a predator (a cat) and those showing no response. However, these two groups did not differ in their responses to a neutral odor, or in the social interaction or elevated plus-maze tests of anxiety. This suggests a distinction between phobic anxiety (generated by cat odor) and a generalized anxiety state (generated by novelty in the other tests). Trial 2 in the plus-maze generates a state of fear that is distinct from that generated on trial 1, and one suggested to reflect phobic anxiety. Although the groups of cat responders and nonresponders did not differ in their scores on trial 2 in the plus-maze, two clear groups of responders and nonresponders could be identified on this trial (but not on trial 1 or in the social interaction test). This suggests that it is possible to identify bimodal populations of rats in tests of both innate and acquired simple phobias.

Simple phobia Generalized anxiety Cat odor

THE Blanchards' group (2–4) described the pattern of behavioral responses of wild and laboratory-bred rats to the presence or odor of a predator (a cat). In laboratory rats, these are innate reactions, because they are shown in animals with no prior experience of a cat or its odor. Even more interestingly, while those that respond to cat odor show no habituation of their behavioral response (3,15,17), not all rats respond to the odor (1,8). Thus, it is possible to compare the neurobiological differences between responders and nonresponders to this predator odor. These groups, defined on the basis of their behavioral response, have been found to differ in their corticosterone response to cat odor (8) and in their concentrations of noradrenaline in the frontal cortex (1).

Blanchard et al. (3) proposed that a rat's response to cat odor was a good model of anxiety, and Zangrossi and File (16) suggested that, in particular, it might reflect a simple phobia. One reason for this suggestion was the lack of specific benzodiazepine effects on number and time of contacts with the odor and the occurrence of sheltering (4,16), at doses that are potentially anxiolytic in other tests of anxiety. The clinical

definition of phobia includes the notion of an irrational fear, but this is with respect to the context and extent of fear, rather than the identity of phobic objects. The list of these is limited and nonarbitrary, frequently features animals, and has a strong genetic component. The irrational aspect of phobia is obviously the hardest to address in an animal test.

The purpose of the present experiment was to determine whether responders and nonresponders to cat odor differed in their responses to a neutral odor or in other animal tests of anxiety. We would not expect any differences if, indeed, the phobic state generated by exposure to cat odor were distinct from the anxiety state induced by other animal tests. We chose to examine their responses in two other tests that use unconditioned responses to ethologically relevant stimuli and probably most closely reflect generalized anxiety disorder [see (11) for review]: the social interaction test (5) and the elevated plus-maze (12). We also examined their responses on trial 2 in the plus-maze. On trial 1, the stimuli generating anxiety are the novelty of the apparatus and the open aspects of the maze (14). However, on trial 2, a different type of anxiety is gener-

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ated (13) and it has been suggested that during the first trial the rats rapidly acquire a fear of heights, and so that by trial 2 the response reflects a phobic response (6,9). We, therefore, wished to determine whether the cat odor responders and non-responders (i.e., those showing differences in an innate phobic response) differed in their behavior on trial 2 in the plus-maze (i.e., in a phobic response that is rapidly acquired).

METHOD

Animals

Male hooded Lister rats (Olac. Ltd., Bicester), approximately 250 g in weight, were singly housed with food and water freely available, in a room maintained at 22°C and with lights on from 0700 to 1900 h.

Apparatus

Exposure to cat odor. The cat odor was obtained by rubbing a damp cloth vigorously against the fur of a laboratory-housed domestic cat for 5 min. The cloth was then kept in a sealed plastic bag until use. Damp pieces from the same original cloth were used as a neutral odor. Odor exposures took place in a small, dimly lit room and before the first cat odor exposure, the cat odor cloth was left in the room for 10 min. Each rat was carried to the test room in its home cage, which was placed next to an empty cage. The cloth was wedged between the two cages, at the end furthest from the food and water containers.

Social interaction test. The test arena was a wooden box 60 × 60 cm, with 35 cm high walls and was brightly lit. A camera was mounted vertically above the arena and the rats were observed from a video monitor in the adjacent room. Infrared photocells were mounted in the walls, 4.5 and 12.5 cm from the floor, and the interruption of these beams provided automated measures of locomotor activity and rearing, respectively. The output from the photocells and the scores of the observer were entered into a microcomputer.

Elevated plus-maze. This apparatus was made of wood and consisted of two open arms, 50 × 10 cm and two opposite closed arms, 50 × 10 × 40 cm with an open roof. The arms were connected by a central square 10 × 10 cm; thus, the maze formed the shape of a plus sign. It was elevated to a

TABLE 1

MEAN (± SEM) TIME SPENT SHELTERING, NUMBER OF CONTACTS, TIME IN CONTACT, AND TIME SCANNING FOR CAT RESPONDERS ($n = 17$) AND NONRESPONDERS ($n = 14$) WHEN EXPOSED TO EITHER NEUTRAL OR CAT ODOR

	Cat Responders	Cat Nonresponders
Cat odor		
Time sheltering (s)	225.9 ± 8.1	41.1 ± 6.2*
Number of contacts	4.7 ± 0.6	13.4 ± 0.9*
Time in contact (s)	18.9 ± 2.9	77.6 ± 5.6*
Time scanning (s)	83.9 ± 8.4	35.2 ± 16.7†
Neutral odor		
Time sheltering (s)	36.0 ± 4.7	31.8 ± 5.5
Number of contacts	12.6 ± 1.0	13.9 ± 1.2
Time in contact (s)	63.3 ± 8.2	64.9 ± 9.8
Time scanning (s)	9.9 ± 1.9	16.5 ± 2.9

* $p < 0.001$. † $p = 0.01$ compared with responder group, analysis of variance.

TABLE 2

MEAN (± SEM) TIME SPENT SHELTERING, NUMBER OF CONTACTS, TIME IN CONTACT, AND TIME SCANNING WHEN EXPOSED TO CAT ODOR, AND TIME SPENT IN SOCIAL INTERACTION, PERCENT NUMBER OF ENTRIES AND PERCENT OF TIME SPENT ON OPEN ARMS OF THE PLUS-MAZE BY RESPONDERS ($n = 19$) AND NONRESPONDERS ($n = 10$) TO CAT ODOR

	Cat Responders	Cat Nonresponders
Cat odor		
Time sheltering (s)	249.9 ± 8.1	74.4 ± 10.5*
Number of contacts	2.5 ± 0.5	12.8 ± 1.5*
Time in contact (s)	14.5 ± 2.7	94.6 ± 13.0*
Time scanning (s)	108.6 ± 14.9	34.9 ± 8.9†
Social interaction		
Total time	52.5 ± 4.7	43.2 ± 3.3
Plus maze Trial 1		
Percent No.	32.1 ± 2.1	34.7 ± 3.3
Percent time	28.9 ± 3.2	30.1 ± 5.1
Plus maze Trial 2		
Percent No.	15.1 ± 3.5‡	8.2 ± 3.9‡
Percent time	10.1 ± 3.0‡	3.1 ± 1.6‡

* $p < 0.001$. † $p < 0.01$ compared with responder group, ‡ $p < 0.001$ compared with plus-maze trial 1 scores, analysis of variance.

height of 50 cm above the floor. A camera was mounted vertically above the central square and the rats were observed from a video monitor in the adjacent room.

Procedure

Experiment 1. Rats were given a 5 min exposure to a neutral odor during which the time spent avoiding the cloth by sheltering under the food and water containers was scored. On the next day, the same rats were given a 5-min exposure, in the same room, to cat odor and the same behavioral measures scored. On the basis of these scores they were divided into responders (>200 s sheltering) and nonresponders (<100 s sheltering), see Table 1.

Experiment 2. Another group of rats was given 5-min exposures to cat odor, as in Experiment 1. On the basis of their times spent sheltering, they were divided into responders and nonresponders, see Table 2. Three days later these rats were given a 4.5-min social interaction test. Immediately after this test, each rat was placed in the elevated plus-maze for 5 min. The next day the rats were given their second 5-min trial in the plus-maze.

RESULTS

The rats were divided into responders and nonresponders to cat odor on the basis of their times spent sheltering. These groups also differed significantly in the number of contacts made with the odor cloth, $F(1, 29) = 68.8$, $p < 0.001$, the time spent in contact with the cloth, $F(1, 29) = 95.1$, $p < 0.001$, and the time spent scanning with head extending out from the shelter, $F(1, 29) = 7.6$, $p < 0.01$ (see Tables 1 and 2). However, the cat responders and nonresponders did not differ in their responses to the neutral odor, in the social interaction test, or in their scores on trial 1 or trial 2 in the plus-maze (see Tables 1 and 2).

The plus maze scores were significantly reduced between trial 1 and trial 2 [percent number; $F(1, 28) = 63.2$, $p <$

0.001; percent time; $F(1, 28) = 58.6, p < 0.001$]. However, although there was no difference on trial 2 in the plus-maze between the cat responders and nonresponders, two clear groups of responders emerged. Thus, 48% of the rats made zero entries on the open arms on trial 2 (plus maze responders); the remainder made a mean of $24.6 \pm 2.7\%$ of entries onto open arms (plus maze nonresponders), and their scores were normally distributed across the range. This bimodal response was not shown either for the scores on trial 1 in the plus-maze or for the social interaction scores, both of which were normally distributed. Although the trial 2 responders and nonresponders did not differ in their responses to cat odor (on all measures $F < 1.0$) or significantly so in the social interaction test [total time; $F(1, 29) = 3.2, p < 0.10$], they did differ significantly in their scores on trial 1 in plus-maze, with the trial 2 responders having significantly lower scores [percent number, $F(1, 29) = 6.5, p < 0.05$; percent time, $F(1, 29) = 7.9, p < 0.01$] (see Table 3).

DISCUSSION

We have previously demonstrated the existence of two distinct populations with regards to the behavioral response to cat odor (1,8), and this study demonstrates that this phenomenon is not affected by preexposure to neutral odor. The results of the present experiment lend further support to the suggestion that exposure to cat odor generates a state of anxiety that is different in kind from that generated by other animal tests and, indeed, different patterns of GABA and 5-HT release and, hence, different neurochemical profiles have already been reported (7,10). Although clear responders and nonresponders to cat odor could be identified, they did not differ in their response to a neutral odor or significantly in their responses in the high light, unfamiliar test condition of the social interaction test, or in the elevated plus-maze (although there was a trend for the cat responders to be less anxious than the cat nonresponders on trial 2 in the plus-maze).

We had selected the most anxiogenic test condition of the social interaction test because, at least as judged by the plasma corticosterone responses, exposure to cat odor was more aversive than other anxiogenic stimuli. While we cannot exclude the possibility that differences might have been found in other test conditions, there is no reason to suppose that this is likely. Testing in the social interaction test prior to plus-maze is a procedure we have frequently used and is similar to holeboard exposure prior to plus-maze (12); we deliberately avoided the

TABLE 3

MEAN (\pm SEM) TIME SPENT SHELTERING FROM CAT ODOR, TIME SPENT IN SOCIAL INTERACTION, PERCENT OF NUMBER OF ENTRIES AND PERCENT OF TIME SPENT ON OPEN ARM OF THE PLUS-MAZE BY RESPONDERS ($n = 14$) AND NONRESPONDERS ($n = 15$) ON TRIAL 2 IN THE PLUS-MAZE

	Plus-Maze Responders	Plus-Maze Nonresponders
Cat odor		
Time sheltering (s)	175.2 \pm 25.8	202.6 \pm 22.7
Social interaction		
Total time (s)	43.3 \pm 2.4	54.8 \pm 5.7
Plus maze Trial 1		
Percent No.	28.8 \pm 2.4	37.0 \pm 2.2*
Percent time	22.3 \pm 2.9	35.8 \pm 3.8†

* $p < 0.05$. † $p < 0.01$ compared with responder group, analysis of variance.

reverse order because prior testing reduces the aversiveness of the unfamiliar arena used in the social interaction test.

Neither the high light unfamiliar condition of the social interaction test, nor trial 1 in the plus-maze provided any evidence for bimodal responses. Overall, the rats showed significantly greater anxiogenic responses in the plus-maze on trial 2 than on trial 1, a phenomenon that is not always found (12), but has been previously reported (13). It was of particular interest that trial 2 in the plus-maze, another test situation that has been suggested to generate a phobic anxiety state (6,9), did result in a clear division of responders and nonresponders. It will, therefore, be possible to explore neurobiological differences between responders and nonresponders on trial 2 in the plus maze. Comparisons between the cat responders and nonresponders and the plus-maze trial 2 responders and nonresponders should allow identification of features common to a phobic responder and any differences may provide insight into the difference between innate and acquired phobias.

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