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Behavioral Stress Response of Genetically Selected Aggressive and Nonaggressive Wild House Mice in the Shock-Probe/Defensive Burying Test

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SLUYTER, F., S. M. KORTE, B. BOHUS AND G. A. VAN OORTMERSSEN. *Behavioral stress response of genetically selected aggressive and nonaggressive wild house mice in the shock-probe/defensive burying test.* PHARMACOL BIOCHEM BEHAV **54**(1) 113-116, 1996.—Genetically selected aggressive and nonaggressive male wild house mice were tested in the shock-probe/defensive burying test. Five distinct behaviors (burying, immobility, rearing, grooming, and exploration) were recorded in two environmental situations: fresh and home cage sawdust. Nonaggressive animals, characterized by a Long Attack Latency (LAL), showed more immobility in both test situations than animals having Short Attack Latencies (SAL), whereas SAL males displayed more defensive burying than LAL ones when tested with fresh sawdust. Testing with home cage sawdust, however, resulted in the same duration of defensive burying in SAL and LAL. These results support earlier findings about the existence of two heritable, fundamentally different strategies to cope with aversive situations. Aggressive (SAL) animals react actively to environmental challenges, whereas nonaggressive animals react actively or passively, depending on the characteristics of the stressful environment. These mouse lines, selected for attack latency, i.e., aggression, may, therefore, be important tools to unravel the genetic architecture underlying the physiological and neuronal mechanisms of behavioral strategies towards stressful events.

Aggression Defensive burying Wild house mice Behavioral strategies Stress

IN NATURAL populations of wild house mice both fast-attacking and slow or nonattacking animals exist (29). Selection experiments proved that this difference in aggression is, at least partly, genetically determined. Male wild house mice were successfully bidirectionally selected for attack latency, which resulted in an aggressive line, characterized by short attack latencies (SAL), and a nonaggressive line, having long attack latencies (LAL) (26). Several studies showed that these selection lines differ not only for attack latency, but also in a wide variety of behavioral and endocrinological traits. For example, SAL males are behaviorally less flexible in both social and nonsocial situations than LAL males (3), but perform better on a two-way active shock avoidance (1). In general, aggressive individuals appear to show an active behavioral response to challenging situations, whereas nonaggressive ones generally adopt a passive behavioral strategy. These selection lines can, therefore, be regarded as displaying alternative, fundamentally different, heritable strategies to cope with environmental demands (2,4).

The aim of this study was to extend the basic idiosyncracy between behavioral strategies in both selection lines to the shock-probe/defensive burying test. In this paradigm animals are shocked by an electrified probe, whereafter they can use either an active behavioral strategy, namely, the pushing of bedding material toward or over the probe (defensive burying), or a passive behavioral strategy, namely, increased immobility/freezing, to cope with the stressor. The degree of burying is both determined by environmental (bedding material), stimulus (shock intensity), and organismic (individual coping style) factors (9,14,25). Two distinct situations were

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tested to manipulate the environmental familiarity of both selection lines: one with home cage sawdust as bedding material, the other with fresh sawdust.

METHOD

Mice

The lines bidirectionally selected for attack latency both originated from a colony of wild house mice (*Mus musculus domesticus*) maintained at the University of Groningen, the Netherlands, since 1971. The SAL males came from the 49th generation of selection; the LAL males from the 23rd through 26th generation. Differences in number of generations between SAL and LAL originated from difficulties in developing the LAL line per se [see (27)] and inequal rates of reproduction with the LAL females producing smaller litters and at a later age. Both selection lines have been shown to behave similarly to freshly caught wild house mice (Van Oortmerssen, unpublished observations) and to survive under natural conditions (29). The mice were housed in Plexiglas cages (17 × 11 × 13 cm) in a room with an artificial 12 L : 12 D cycle (lights on at 0030 h). Food (standard laboratory chow; Hope farms AM2) and water were available ad lib. Mice were weaned at 3–4 weeks of age, and paired male–female at the age of 6–8 weeks.

Behavioral Testing

Aggression. The aggression test has been extensively described by Van Oortmerssen and Bakker (26). Briefly, the time it takes for a given animal to attack a standard opponent (MAS-Gro) was measured on 3 consecutive days. The attack latency score (ALS) is the mean of these daily scores. Attack latency is a reliable indicator of aggression, because there is a significantly negative correlation between attack latency and the number of attacks and accumulated attacking time, including chasing, biting, and fighting (7,30). Standard opponents should elicit offensive behaviors from the animal to be tested, but not initiate offensive behaviors themselves (10). The opponents used in this study had been attacked and had shown defensive behavior in previous tests; they can be compared to the submissive intruders described by Brain et al. (5).

Only nonattacking LAL and SAL males with ALS shorter than 50 s were used.

Defensive burying. The shock-probe/defensive burying test was performed in an identical cage as their home cage. The floor was either covered with fresh or home cage sawdust. A removable Teflon probe (4 cm long, 1 cm in diameter) was placed 1 cm above the floor, inserted through a small hole in the center of the wall of the Plexiglas cage. Two exposed wires (0.5 mm in diameter) were each wrapped (25 times) independently around the probe. Whenever the animal touched the probe with some part of its body an electric current (1.5 mA) was delivered to the animal.

The behavior of the animals during the presentation of the probe was recorded and classified into five elements: defensive burying, immobility, rearing, grooming, and exploring. The duration of appearance of each of these elements was expressed as the percentage of total time of the observation period. Recording started after the first shock and lasted 5 min. The shock circuit was left on during the entire period [repeated shock-probe procedure as described by Treit and Fundytus (24)]. Thus, the procedure investigated the consequence of the direct effect of shock. Using exclusively males, all animals were tested only once.

Statistics

Behavioral data were analyzed using the (nonparametric) Mann–Whitney *U*-test. A probability level of $p < 0.05$ was taken as being statistically significant.

RESULTS

The five distinct behaviors (burying, immobility, rearing, grooming, and exploration) are shown in Fig. 1. Comparisons are presented as home cage vs. fresh sawdust per genotype and SAL vs. LAL per environment.

Home Cage vs. Fresh Sawdust

For all recorded behaviors SAL males tested with home cage sawdust showed similar values to SAL males tested with fresh sawdust. LAL males tested with fresh sawdust showed more immobility ($U = 14.0$, $p < 0.01$) and less burying ($U = 22.5$, $p < 0.05$) than the ones tested with home cage sawdust, whereas no change occurred in the display of other behaviors.

SAL vs. LAL

In the home cage environment LAL males displayed higher levels of immobility ($U = 6.0$, $p < 0.001$) and grooming ($U = 15.5$, $p < 0.01$), whereas SAL males showed higher levels of rearing ($U = 14.0$, $p < 0.01$).

In the fresh sawdust environment SAL animals showed higher percentages of burying ($U = 0$, $p < 0.001$) and rearing ($U = 16.0$, $p < 0.01$), whereas LAL animals showed higher percentages of immobility ($U = 0$, $p < 0.001$).

DISCUSSION

The results support earlier theories about the existence of two different behavioral strategies to cope with a stressor in wild house mice. Nonaggressive, LAL males preferentially adopt a passive strategy, i.e., immobility/freezing, in a stressful environment (fresh sawdust); however, LAL males are also able to adopt an active strategy, i.e., defensive burying, when the environment is less aversive (home cage sawdust). Aggressive, SAL males, though, do not shift their behavior whether exposed to fresh or home cage sawdust. Independent of the qualities of the environment, SAL males display defensive burying, i.e., an active behavioral strategy. This more routine-like behavior of SAL animals is in line with earlier findings. When tested for behavioral flexibility in a Y maze in which a blocked arm was reversed, SAL males show higher percentages of errors, i.e., are more routine-like than LAL males (3,23).

Accordingly, in addition to previously found behavioral dissimilarities [summarized by Benus et al. (2)], SAL and LAL males also show differences in the shock-probe/defensive burying test. The fact that aggressive males act actively and nonaggressive ones either actively or passively in the shock-probe/defensive burying test is in agreement with earlier results with wild-type rats, for which a significant correlation was found between attack latency and the amount of time spent in burying the probe (17).

The idea of two, fundamentally different, heritable strategies in which behavioral, neuronal, and physiological characteristics are genetically correlated, is supported by observations in other rodent selection lines. Mouse lines selected for high thermoregulatory nest-building behavior are more aggressive than their low nest-building counterparts (20). One may put different labels to this idiosyncracy like emotionality or emotional reaction (6,11), differential use of internal and external information (8), aggressive and timid [e.g., (15), or active and passive coping (2,4). Important is that the extremes of a natural variation have distinct behavioral, pharmacological, physiological, and neuroendocrinological profiles, result-

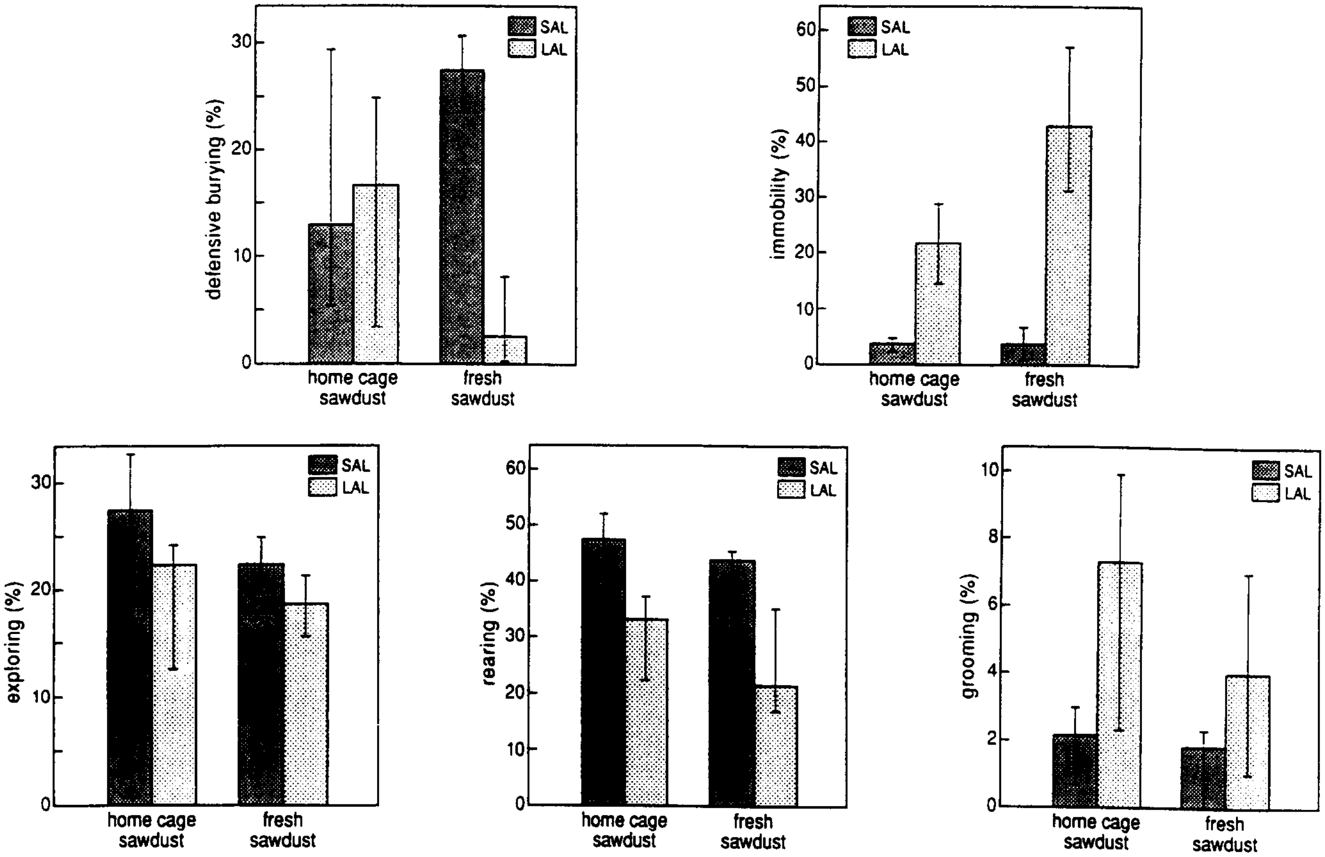

FIG. 1. Percentages of time spent of aggressive SAL and nonaggressive LAL males on the five distinct behavioral measures (burying, immobility, rearing, grooming, and exploration) in two differentially aversive environmental settings: fresh and home cage sawdust. For each group 10 males were used. Percentages are expressed as medians and quartiles.

ing in two different types of individuals within a species. The neuroendocrinological consequences of these behavioral strategies differ substantially: the immobility/freezing response is accompanied by a predominant activation of the HPA axis and the adrenomedullary system, resulting in pronounced elevations of corticosterone and adrenaline, respectively, whereas the defensive burying response is accompanied by a preferential neurosympathetic activation, resulting in increased plasma noradrenaline levels, tachycardia, and hyperthermia (9,14). In this respect, it is noteworthy to mention that rats selected for superior shock avoidance acquisition (RHA/Verh) show more defensive burying than those selected for inferior shock avoidance acquisition (RLA/Verh) after administration of noradrenaline in the central amygdala (16). Furthermore, the behavior of rodents in the shock-probe/defensive burying paradigm appears to be particularly sensitive to anxiolytic drugs of both the classical benzodiazepine and novel serotonergic ones (9,13).

Whether this difference in behavioral strategies in SAL and LAL is purely genetic remains to be investigated. Selection lines do not only differ for genetic (autosomes, X and Y chromosome, mitochondrial DNA) factors, but also for environmental factors, including cytoplasmic and maternal factors. Genomic imprinting can also not be excluded. However, if coping behavior is as closely related to aggression, as suggested by prevous studies, general and postnatal maternal environmental effects are not very likely. Both embryo transfer of SAL and LAL morulae and blastocysts to similar recipients (18) and crossfostering (21) do not affect aggression in SAL and LAL and their reciprocal F_1s at adult age. One important genetic factor and potential subject of subsequent studies is the Y chromosome. Strong indications have been found for a Y chromosomal involvement in the development of differential aggression (22,28), whereas Y chromosomal effects on differential sizes of the hippocampal intra- and infrapyramidal mossy fiber terminal fields (12) and apomorphine-induced stereotyped behavior (19) have been demonstrated with certainty in wild house mice.

Summarizing, wild house mice were selected on attack latency, i.e., aggression, and were tested in the shock-probe/defensive burying test paradigm. The aggressive animals respond actively, whereas nonaggressive animals act, depending on the environment, either actively or passively. This differential expression of behavioral measures in reaction to environmental challenge, i.e., defensive burying (SAL) and immobility (LAL), may be an important tool to investigate both the effects of anxiolytic drugs and the genetic, physiological, and neuronal mechanisms of stress and anxiety.

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