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Strain Differences in Fear-Motivated Behavior of Rats

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REX, A., U. SONDERN, J. P. VOIGT, S. FRANCK AND H. FINK. *Strain differences in fear-motivated behavior of rats.* PHARMACOL BIOCHEM BEHAV **54**(1) 107–111, 1996.—Studies have shown different and sometimes contradictory results in response to anxiolytic drugs. In the present study, the behaviorial performance of rat strains, obtained from different breeders, was examined in four animal models of anxiety- or in exploration-related behavior to assess the potential contribution of genetic disposition or breeding factors to aversion-motivated behavior. Male rats: Wistar/Winkelmann, Wistar/Charles River, Wistar/BGVV, Lewis/Charles River, Fischer/Charles River, Brown Norway/Charles River were used in a conflict test in the open field, a free exploratory paradigm, social interaction test, and the holeboard test. The results show that robust behaviorial differences in anxiety or exploration exist between different strains of rat and animals of one strain, obtained from different breeders. The differences shown in anxiety-related behavior might explain sometimes contradictory effects following the treatment with anxiolytic or anxiogenic drugs. The results indicate that genetic factors and breeding conditions substantially contribute to anxiety-motivated behaviors in animal models of anxiety. These differences in anxiety-related behavior may also be related to biochemical differences.

Anxiety Strain differences Exploration Holeboard Conflict test Social interaction

ANXIETY is an emotional state experienced by humans, and is not readily modeled in animals. Nevertheless, a need exists for animal models that may be useful for prediction of the anxiolytic properties of drugs for potential clinical use and as test systems for studying the neuronal transmission systems and mechanisms involved in the development of anxiety (12,28). A wide range of tests has been developed for this purpose (17,21,27). Most of the tests are based on the ability of anxiolytic agents to increase the frequencies of behaviors that have been suppressed by punishment, by social threat, or by exposure to natural threats such as light environments or open spaces.

Although the traditional tests of anxiolytic activity depend on the release of punished behavior [e.g., (15,30)], more recently developed anxiety tests make use of the native behaviorial neophobia of rodents (22).

Previous studies have shown different and sometimes contradictory results in response to anxiolytic drugs using the same animal model of anxiety and test procedures [e.g., (11)]. Especially, nonbenzodiazepine anxiolytic drugs [e.g., (4,6,18)] failed to be effective in several animal models, such as 5-HT_{1A} agonists (7) or 5-HT_3 antagonists in the elevated plus-maze and the social interaction test (14). There are some explanations for inconsistent findings in measures of anxiety in different laboratories. Beside the nonoptimal environmental conditions in the laboratory (32), it is known that baseline behavior is markedly affected by various determinants, for example, prior stress and handling, lighting conditions, pretesting, and housing (8,25). Anxiety- and fear-related behavior is affected by gender and age of the animals used (12). Additionally, strain differences have been shown to cause distinct results in anxiety-related behavior (5,16,24,29).

Differences in the behaviorial baseline in animal models of anxiety or exploration may be a critical determinant of response to anxiolytic drugs (25).

In the present study, the behaviorial performance of rat strains obtained from different vendors was examined in three animal models of anxiety and in one model of habituation/exploration related behavior to assess the potential contribution of genetic disposition or breeding factors to aversion-motivated behavior. The animal models used were: a) a conflict test in the open field, based upon the suppression of feeding by exposure to a novel and aversive environment (1,3); b) a free exploratory paradigm (19) allowing the animals to explore the unknown surroundings starting from the familiar home cage; c) the social interaction with an unfamiliar partner (10,13) under high light conditions in the unfamiliar open field; d) the holeboard apparatus (2,9,31) for the evaluation of exploration and habituation.

Aims of the study were the determination of strain differ-

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ences in anxiety-related behavior in naive rats and the investigation of the suitability of rat strains in behaviorial tests.

METHOD

Animals

Male rats 250 ± 30 g (age 7-11 weeks), from different strains and obtained from different vendors and breeders in Germany were used:

strain	*vendor*	*strain-short name*	*abbreviations*
Wistar	Winkelmann	BOR: WISW	WW
Wistar	BGVV	HAN: WIST(SYN WI)	WB
Wistar	Charles River	CRL: (WI)WU BR	WC
Lewis	Charles River	LEW/CRLBR	LC
Fischer	Charles River	CDF (F-344) /CRLBR	FC
Brown Norway	Charles River	BN/CRLBR	BC

All animals were group housed, five rats per cage (45 × 60 × 25 cm), at room temperature (22°C) and under a 12 L : 12 D cycle (lights on at 0600 h). Standard pellet food (Altromin 1326) and water were freely available.

All rats were used in the four tests with 1 week interval between tests. The animals were not handled between the tests to avoid handling-induced effects on fear-related behavior. The tests were performed in the same order for all strains/lines of rats. The tests were performed on 2 days, and rats from different strains or vendors were assigned randomly to the procedure. Group size: n = 10–11.

The experiments were performed in a sound proofed, observation chamber between 0900 and 1100 h.

Conflict Test

Twenty hours before testing the food was removed from the animal's home cage, with water still available. One hour prior to testing the animals were transferred into the brightly illuminated (≈1000 lx) observation chamber. The test was performed in a white wooden open field (100 × 100 cm × 40 cm). The usual food pellets were placed in the center of the open field.

The animals were placed individually in one corner of the open field. Each rat was observed for 5 min and the latency to the initial feeding was recorded. The incidence of rats taking food [% of rats in a group feeding] was registered.

Locomotor Activity

Locomotor activity was assessed simultaneously during the conflict test (5 min) by interruptions of 10 equally spaced infrared light beams in the open field.

Free Exploratory Paradigm

The free exploratory paradigm was performed in the familiar surroundings of the animal unit. The lid from the animal's home cage was removed. The cage cover and the number of animals exploring outside the home cage during the first 10 min was recorded [% of rats in a group].

Social Interaction Test

Twenty-four hours before the experiment the rats of the established groups were separated and kept in single cages overnight. The next day the rats were placed in an unfamiliar brightly illuminated (≈1000 lx) white open field (100 × 100 × 40 cm) with an unfamiliar rat taken from a different group. Each pair of rats was observed for social contact for 10 min and the time of contact determined (s).

Holeboard Test

The animals were placed in a white wooden box (50 × 50 × 30 cm) with a floor containing 16 equally spaced holes (2.5 cm diameter, 10 cm apart). The number of head dips was registered automatically by infrared light beams. The tests were performed on 2 consecutive days exposing the rats to the holeboard for 10 min and the number of head dips were recorded. A reduction of head dips on the second day was interpreted as habituation in an unfamiliar environment (ratio of head dips second/first day in %).

Statistics

The data from the conflict test and the free exploratory paradigm were expressed as percent and were analyzed using the χ^2-test. Locomotor activity data, data from the social interaction test, and the holeboard test (means ± SEM) were analyzed using the one-way ANOVA followed by a multiple comparison with the Student-Newman-Keuls test. Differences with $p < 0.05$ were considered as statistically significant.

RESULTS

Locomotor Activity

The measurement of locomotor activity in the open field revealed considerable interstrain differences in rats acquired from one breeder: LC: 101 ± 11, FC: 36 ± 8, BC: 78 ± 7, WC: 108 ± 11 (squares crossed in the open field, means ± SEM). Similar differences in locomotor activity were to be seen in Wistar rats obtained from different breeders and vendors: WW: 129 ± 12,WC: 108 ± 11, WB: 48 ± 9 (squares crossed in the open field, means ± SEM).

Conflict Test

In the conflict test rat strains the number of rats feeding during the 5-min test session had a substantial interstrain vari-

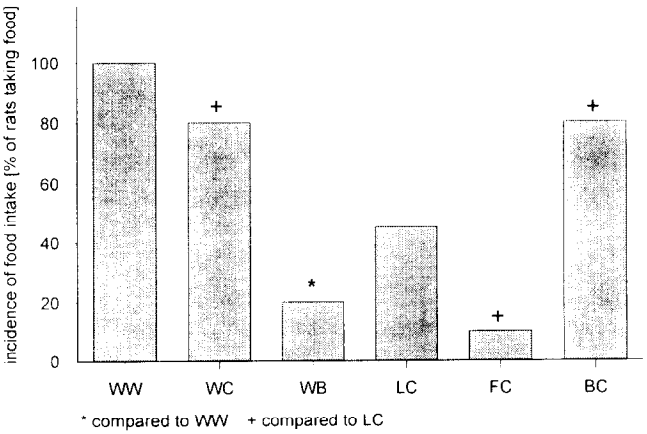

FIG. 1. Alterations in the incidence of food intake in the open field. The data are expressed as percent and were analyzed using the χ^2-test (n = 10). Differences between the groups with $p < 0.05$ were considered as statistically significant (*differences between the different lines of wistar rats, +differences between different strains from one vendor).

ability (Fig. 1). The various Wistar rats showed a similar variation in the percentage of rats feeding in the aversive open field (% of animals in a group) (Fig. 1).

Free Exploratory Paradigm

In the free exploratory paradigm the rats from different strains displayed a nonforced exploratory behavior (% of animals exploring novelty) with strain differences corresponding to the forced exploration in the conflict test (Fig. 2). Free exploratory activity in the different Wistar rats had a variation of the same magnitude as in the different strains obtained from one breeder, with the Winkelmann Wistar rats showing a decreased exploration compared to the conflict test (Fig. 2).

Social Interaction Test

In the social interaction test the amount of time the animals spent in active social contact was increased in the Lewis rats compared to the other rat strains. In the other rat strains there was no significant difference in the time of contact. Compared to the various Wistar rats the BGVV-Wistar rats spent less time in active contact (Fig. 3).

Holeboard Test

In the different strains the total number of head dips in the holeboard test during the first trial showed no significant interstrain differences. The Winkelmann Wistar rats had a higher explorative activity than the Charles River Wistar rats (with the BGVV rats not tested). Comparing the habituation determined by the ratio of head dips second/first trial there was no marked difference between the various strains and the two different Wistar rats (Fig. 4).

DISCUSSION

The results show that robust behaviorial differences in anxiety or exploration exist between different strains of rats and animals of one strain obtained from different breeders. To exclude the possibility of batch variation, different batches from two different lines of Wistar rats (Winkelmann and BGVV) and Brown Norway rats were used several times in the test battery, showing constant behavioral profiles. The differences shown in anxiety-related behavior using untreated rats might explain the sometimes contradictory effects following the treatment with anxiolytic or anxiogenic drugs seen in the literature (7,18). The animal models used exploit a different kind of stimuli, for example, a forced exploration of a novel aversive environment in the conflict test, a voluntarily exploration of the environment in the free exploratory para-

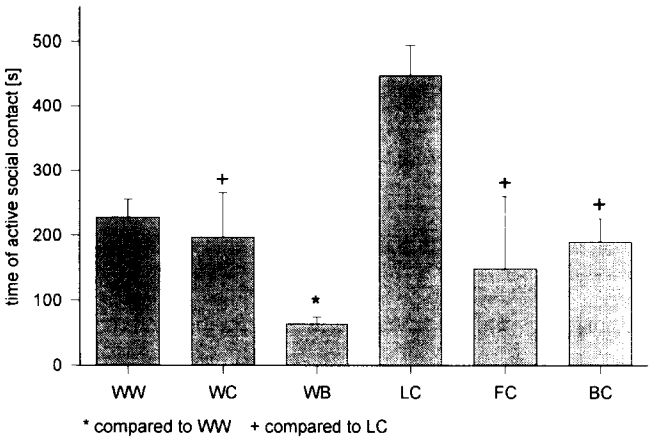

FIG. 3. Time spent in active social contact between two nonfamiliar rats in the open field. Data are expressed as means ± SEM analyzed using the one-way ANOVA followed by a multiple comparison with the Student-Newman-Keuls test ($n = 10$). Differences between the groups with $p < 0.05$ were considered as statistically significant (*differences between the different lines of Wistar rats, +differences between different strains from one vendor).

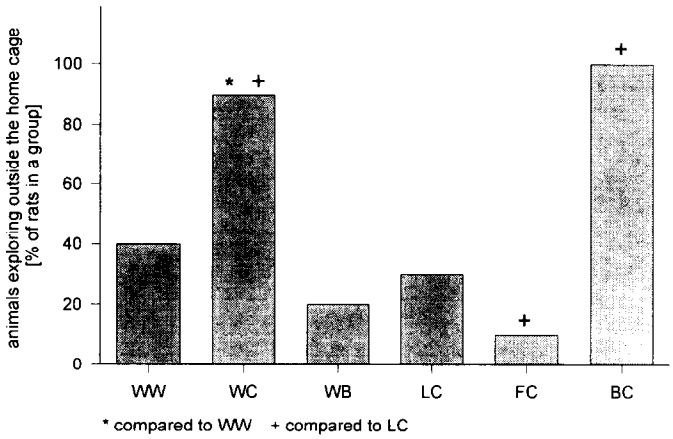

FIG. 2. Free exploratory behavior under familiar conditions. Data showing the percentage of rats exploring surroundings outside the home cage and were analyzed using the χ^2-test ($n = 10$). Differences between the groups with $p < 0.05$ were considered as statistically significant (*differences between the different lines of Wistar rats, +differences between different strains from one vendor).

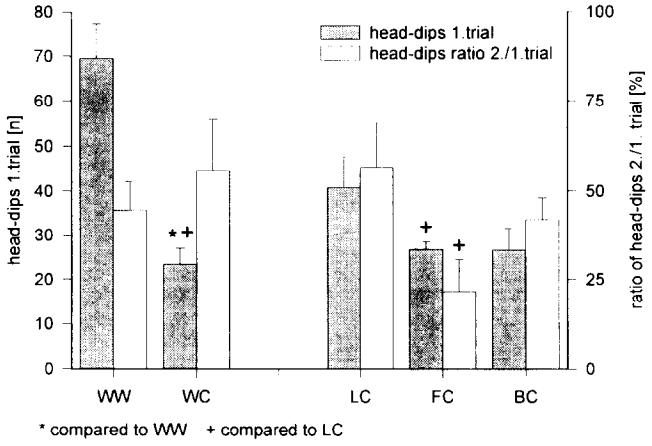

FIG. 4. Habituation of exploratory behavior in the holeboard. Data showing the total numbers of head dips on 2 consecutive days and the ratio of head dips from the second/first day in percent as a measurement of habituation. Data are expressed as means ± SEM analyzed using the one-way ANOVA followed by a multiple comparison with the Student-Newman-Keuls test ($n = 10$). Differences of the means with $p < 0.05$ were considered as statistically significant (*differences between the different lines of wistar rats, +differences between different strains from one vendor).

digm, the social interaction test, and the exploration and habituation in an aversive environment in the holeboard test.

Differences seen in the fear-related behavior are not related closely to locomotor activity, because the anxiolytic effects in rat strains differ, despite a similar locomotor activity in the open field (e.g., LC and WC). The distinct differences in fear-related behavior between rat strains or lines cannot be explained by changes in locomotor activity.

The results indicate that genetic factors substantially contribute to anxiety-motivated behaviors in animal models of anxiety as well as environmental factors and breeding conditions. These differences in anxiety-related behavior may be related to existing biochemical differences between rat strains, e.g., between Fischer and Lewis rats (20,23,26). Based on the known connection between fear/anxiety and the central serotonergic system, we suspect differences between the strains or lines of rats either in the serotonin release or in the receptor distribution. The analysis of the genetic differences and the subsequent biochemical differences between rat strains displaying distinct behavior in animal models of anxiety may lead to better knowledge of the neurochemical basis of anxiety-related behavior.

The use of different strains in animal models of anxiety may be an effective way to assess genetic contributions to specific behavior. Biochemical differences between rat strains possibly causing differential behaviorial profiles should be taken into account. There is the possibility that differences in the behavior of rats are partially caused by breeding conditions and, therefore, it is sometimes difficult to duplicate the findings from other labs, even if the same strain of rats has been used. Genetic differences between rat strains are well documented [e.g., (23,26)]. However, there is a lack of data on intrastrain differences and the possible influence of environmental conditions maintained by the breeder and vendor on anxiety-related behavior.

Further work is required on a similar approach, but using animals from different strains raised in our animal unit to exclude differences induced by the breeding conditions and to investigate the effects of laboratory manipulations on strain differences.

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