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TEMPORAL CHARACTERISTICS OF GROOMING IN AN OPEN FIELD IN TWO STRAINS OF RATS

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ABSTRACT: The temporal characteristics of grooming in an open field were studied in rats from two different genotypes (NR, brown Norway rats bred from an original wild stock and KM, Krushinsky-Molodkina albino rats selectively bred for audiogenic seizure susceptibility). The measures of grooming recorded were time of onset of any grooming activity, duration and number of grooming episodes and total time spent grooming during successive 3-min intervals over a total 12-min period. The results demonstrated that grooming episodes of different durations displayed different features across the course the test. Grooming was minimal in the first minutes of the test and the longest grooming episodes were observed after the sixth minute in most of the rats. The number and proportion of prolonged episodes (over 21 s in duration) increased over time. Short-duration episodes (1-3 s) were not connected with the specific stage of the test and/or the decrease in locomotion. The scores of grooming duration were higher in NR in comparison to the KM rats. No significant effects were found for strain and sex for total numbers of grooming episodes.

Grooming behavior in rodents, and particularly in the rat, is often observed as an expression of stress in novel, dangerous or conflict situations (Barnett, 1975; Bindra & Spinner, 1958; Cohen & Price, 1979; Fentress, 1973; Jolles, Rompa-Barendregt, J. & Gispen, W. H., 1979). It has also been identified as a displacement activity (Delius, 1970; Krushinsky & Semiokhina, 1973; Tinbergen, 1952). Although grooming behavior has been studied extensively (see Berridge, 1990), there are relatively few studies of grooming on the open field, and in such studies (e.g. Doyle & Yole, 1959; Ivinskis, 1968; O'Kelly, 1949) observations were typically made for only two or three minutes. Prolonged exposure to the open field induces habituation to novel or fear-inducing factors, and so their stressful effects likely decrease over the course of time. Observations indicate that there is a trend toward a

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high level of grooming behavior with prolonged exposure to the open field.

In order to understand what mediates the grooming response, we measured the time of onset and duration of grooming episodes, as well as the relationship of grooming with other behaviors on the open field across a 12-min period. The behavior of two stocks of rats was compared: brown Norway rats, with high grooming scores and a rich behavioral repertoire; and rats from the Krushinsky-Molodkina stock (albino rats selectively bred for susceptibility to audiogenic seizures) with poor grooming scores (Pleskacheva, Sotskaya, Krushinsky et al., 1990). Shtemberg (1982) and Pleskacheva (1985) have reported an increase in grooming duration in the brown Norway rat after the third minute of observation in the open field. And Pleskacheva et al. (1990) observed no decrease in time spent grooming by Krushinsky-Molodkina rats during a second open-field trial.

METHOD

Subjects

Two strains of rats were studied. Albino rats from the Krushinsky-Molodkina (KM) stock and brown Norway rats (NR). The Norway rats were from the tenth generation of an original wild stock. The albino rats were selectively bred for audiogenic seizure susceptibility; they exhibited clonic and tonic seizures within 1.5 min of exposure to the sound of a 120db electric bell (Krushinsky, Molodkina, Fless, et al., 1970; Semiokhina, Fedotova & Kuznetsova, 1993). In comparison with commercially outbred albino rats that do not show audiogenic seizures, serotonin and norepinephrine levels are reduced in this stock (Sergienko & Loginova, 1983), cortical acetylcholinesterase activity is high (Eremeev, 1969), and there are deficits in the binding capacity of GABA and benzodiazepine receptors in the cerebellum, neocortex and brain stem (Zhulin & Pleskacheva, 1991).

Twenty-seven females and 27 males of the KM strain, ranging in weight from 200-230 gm and 300-350 gm respectively, and 26 females and 26 males of the NR strain, ranging in weight from 200-250 gm and 300-400 gm respectively, were observed. Both stocks ranged in age from 4 to 8 months, were experimentally naive, had received no previous handling, and had not been bred. Rats were weaned when one month old. Three to five rats (usually from the same litter) of the same sex, age and stock were housed in plastic cages (40 x 35 x 16 cm) in a

colony room maintained on a 10:14 light/dark cycle and at 20 degrees Celsius. They were fed on a diet of grain, bread, boiled and raw meat, cabbage and carrot. Food and water were freely available.

Apparatus

The animals were tested in a circular open field, 100 cms in diameter. The 50 cm high walls were made from light tin and the floor was covered with light brown linoleum, divided into 16 sections by black grids (22x22 cm): the 4 central squares were of equal area, the other 12 segments were somewhat smaller. The field was illuminated by a 200 watt bulb, centered above the apparatus, 50 cm above the floor. Noise in the experimental room was from 30-40 db.

Procedure

The animals were tested during the light phase of their L/D cycle, between 10 a.m. and 3 p.m. When removed from the home cage a rat was placed on the experimenter's arm and transferred to the centre of the open field where it was observed for 12 minutes. Each rat was tested once. The apparatus was cleaned with a very weak soapy solution between subjects.

The following measures of grooming were recorded using a stopwatch and checklist: time of onset of any grooming activity (washing muzzle and head area behind the ears, body licking, anogenital and tail grooming, scratching); duration of each grooming episode (minor interruptions of 1-2 s were ignored); number of grooming episodes; and total time spent grooming during successive 3-min intervals and for the entire 12-min observation period. The observer also recorded the number of floor squares crossed; rearing (number of vertical postures); urination (presence or absence) and defecation (number of fecal boli).

RESULTS

General characteristics of rat grooming

Figure 1 shows the mean time spent grooming by males and females of both strains of rats across successive 3-min blocks of the 12-min observation period. With the exception of one KM rat, all rats were observed to groom during the open-field test. Although both

strains exhibited distinctive grooming activities, which will be described below, several features of the grooming patterns were similar in both strains of rats. For example, no grooming occurred during the first test minute; grooming began to appear during the 2nd - 3rd minutes, and increased as time went on. There were considerable individual differences in the number of grooming episodes and, particularly, in the duration of these episodes. These ranged from 1-2 sec of head washing movements to protracted reactions (up to 2.5 min) that included the whole sequence of grooming acts. There was a significant increase in grooming duration in the course of the 12-min open field test. A 2-way repeated measures ANOVA (time by sex) showed this finding for both strains: $F(3,200) = 11.84$, $p<0.001$ for Norway rats and $F(3, 208) = 14.14$, $p<0.001$ for the KM strain. There was no significant effect of sex and no significant interaction.

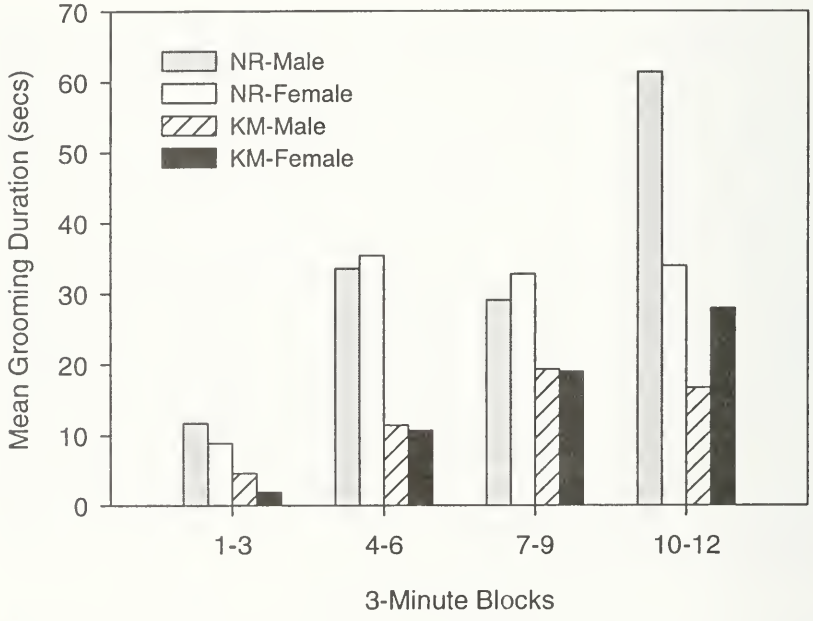

Figure 1. Mean time spent grooming by males and females of the NR and KM strains of rats over the 12 minute observation period.

Grooming was minimal in the first three minutes of the test, and during this period other activities occurred at their maximal value. Table 1 compares activity level (mean number of squares crossed) during the first and last 3-min blocks of the 12-min open-field test. Defecation occurred mainly during initial minutes of the test.

Table 1. Mean squares crossed during first and last 3-min blocks of open field test.

Parameters	NR		KM	
	Males	Females	Males	Females
Minutes 1-3	24.04	35.42	34.77	38.81
Minutes 10-12	9.87	17.85	13.73	12.58

The distribution of grooming episodes of different durations is shown in Figure 2. It is clear that for all groups of rats most episodes of grooming were 3-sec or less in duration.

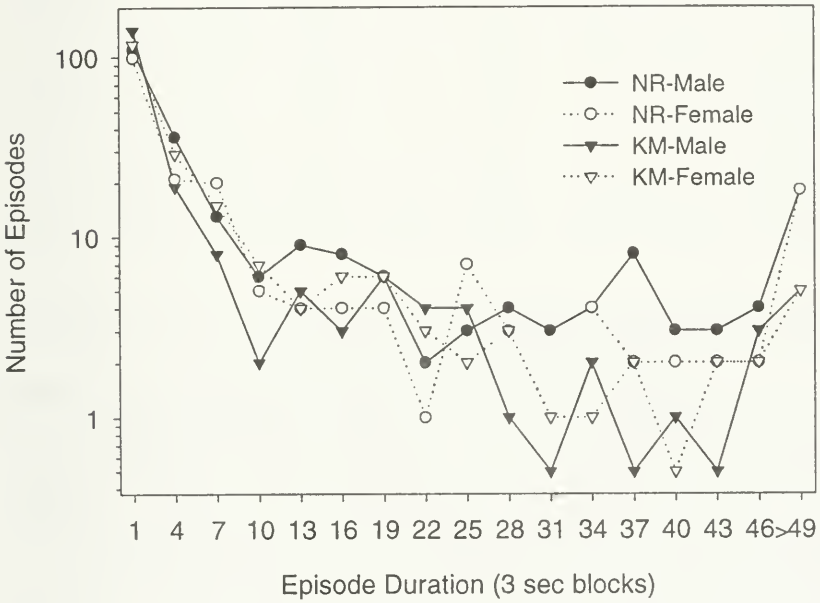

Figure 2. Number of grooming episodes (plotted on a log scale) of different duration in the two strains of rats for each sex. NR, brown Norway; KM, Krushinsky-Molodkina albino rats selectively bred for audiogenic seizure susceptibility.

The percentage of grooming episodes of different durations during each 3-minute block of the 12-minute test for the four groups of rats was also examined. There is an increase in the number of long grooming episodes late in the test. Table 2 shows the results of a

Chi-square analysis comparing frequencies of grooming episodes of different durations. A significant increase in number of episodes was observed only after the third minute. The longest grooming episodes were observed after the sixth minute in most of the rats (from 71% to 91% in different groups).

Table 2. Results of Chi-square tests of the frequencies of grooming episodes during four ranges of durations (1-3 s, 4-6 s, 10-21 s, over 21 s) when each 3-min open field period is compared with each other.

Open Field Period (mins)	Brown Norway Rats				KM Rats			
	Males		Females		Males		Females	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
1-3 & 4-6	1.86	n.s.	7.28	n.s.	7.86	<.05	5.54	n.s.
1-3 & 7-9	4.04	n.s.	13.97	<.005	8.21	<.05	9.72	<.025
1-3 & 10-12	6.70	n.s.	8.63	<.05	8.70	<.05	8.14	<.05
4-6 & 7-9	1.80	n.s.	5.82	n.s.	8.01	<.05	1.57	n.s.
4-6 & 10-12	4.48	n.s.	1.47	n.s.	20.28	<.001	5.08	n.s.
7-9 &10-12	6.73	n.s.	1.94	n.s.	6.51	n.s.	8.85	<.05

Strain and sex differences in grooming behavior

Table 3 shows performance on other behavioral measures for the total observation period (12 min). There were strain differences in some open field parameters. A two-way repeated measures ANOVA (strain x sex, 12 min observations) revealed significant strain effects in rearing ($F=12.7$, $p<.001$), defecation ($F=10.8$, $p<.002$), total duration ($F=44.1$, $p<.001$) and maximum value of grooming ($F=29.8$, $p<.001$). A statistically significant effect for sex was obtained only for number of squares crossed ($F=8.4$, $p<.005$) and defecation ($F=11.9$, $p<.002$). The NR reared more KM rats, but their defecation scores were lower. Number of crossed squares was lowest in NR males. The scores for grooming duration and maximum values of grooming episodes in the open field were higher in Norway rats in comparison to the KM rats. On the contrary, no significant effects were found for strain and sex scores for total numbers of grooming episodes.

Table 3. Mean and (S.E.M.) behavioral patterns of Norway rats (NR) and Krushinsky-Molodlina (KM) rats in an open field over the total 12-min observation period.

Behavioral Pattern	NR Rats		KM Rats	
	Males	Females	Males	Females
N	26	26	27	27
No. of Squares Crossed	68.1 (5.3)	101.5 (7.6)	88.7 (7.4)	97.9 (9.1)
Rearing	25.6 (2.6)	30.6 (2.5)	20.9 (2.6)	19.3 (1.9)
No. of Fecal Boli	3.9 (0.5)	3.4 (0.5)	8.0 (0.8)	3.7 (0.7)
Grooming: Total Duration	136.4 (13.6)	111.1 (11.6)	54.0 (5.2)	59.6 (7.6)
Grooming: Maximum Value	69.7 (7.0)	60.5 (7.7)	34.7 (4.3)	31.8 (4.1)
No. of Episodes	9.4 (1.0)	7.6 (0.8)	7.8 (0.8)	7.6 (0.8)

Table 4. Results of t-tests of strain and sex differences in total grooming durations for Norway rats (NR) and Krushinsky-Molodkina rats (MK) in an open field over successive 3-min periods.

Period (mins)	Strain Differences				Sex Differences			
	Males		Females		NR		KM	
	t (51)	*p*	t (51)	*p*	t (50)	*p*	t (52)	*p*
1-3	2.2	<0.04	3.2	<.004	-	n.s.	2	<.05
4-6	5	<.001	3.4	<.003	-	n.s.	-	n.s.
7-9	-	n.s.	-	n.s.	-	n.s.	-	n.s.
10-12	4.3	<.001	-	n.s.	2.3	<.022	-	n.s.

The results of t-tests of strain and sex differences in total grooming durations are shown in Table 4 (t values are given only for the significant differences). Strain differences in grooming durations

appear in the first minutes of the test, reaching a maximum at the 4^{th}- 6^{th} minute; there were additional large increases in grooming for NR males and a slight increase for KM females during the 10^{th} - 12^{th} minutes (Figure 1 and Table 4). Sex differences were observed at the 10^{th} - 12^{th} min in the Norway rats, with males' scores higher than those of the females. Similar differences were shown by the KM rats during the first test minutes.

There were significant strain differences in the distributions of grooming episodes of different durations across the open field test (Table 5). For the males, these differences were observed in all 3-min intervals except the 7^{th} - 9^{th} -min block. On the contrary, for females significant differences were found in 7^{th} - 9^{th} min period only. The strain differences in distributions of grooming episodes are associated with a greater number of prolonged episodes (22 sec and more) and with fewer of the shortest episodes (1-3 sec) in brown Norway rats. The increase in grooming activity in NR's at 4^{th} - 6^{th} min coincided with a reduction in the number of squares crossed without a decrease in rearing activity. On the contrary, the rearing scores of the KM rats were slowly decreasing.

Table 5. Strain differences in the distributions of grooming episodes across the open-field test (successive 3-min blocks and total scores).

Periods (mins)	Males		Females	
	χ^2	p	χ^2	p
1-3	11.84	<.01	7.06	n.s.
4-6	13.1	<.005	4.34	n.s.
7-9	0.72	n.s.	16.23	<.001
10-12	17.67	<.001	0.8	n.s.
Total:1-12	25.42	<.001	8.97	<.05

DISCUSSION

Grooming by rats in the open field has not been investigated as much as other behaviors such as walking, rearing, and defecation (Walsh & Cummins, 1976). Moreover, scant information on grooming in wild Norway rat and especially Krushinsky-Molodkina rats makes comparison of our results with those of other authors difficult. The

present results confirm strain differences in grooming performance in rats — our data on grooming duration are in agreement with those of Price and Huck (1976), who found more prolonged grooming, especially body grooming, in wild rats in comparison with domesticated Long-Evans rats. As well, Shtemberg (1982) has shown that in brown rats the number of grooming episodes is higher than in Wistar rats.

A relatively high level of emotionality in the KM rats is assumed because they showed high defecation, low rearing, and a low amount of general activity (low scores for number of squares crossed). This finding is compatible with scores for fearful brown Norway rats (Walsh & Cummins, 1976). Undoubtedly there are strain peculiarities in grooming behavior in both sexes. Increased grooming in male Norway rats has been described by Price and Huch (1976), while in the long-Evans strain sex differences were the reverse (Ivinskis, 1968; Price & Huch, 1976). In the present investigation sex differences in the grooming reaction were weaker than strain differences and practically absent in the KM rats.

Recent studies emphasize the importance of temporal profiles for the estimation of open field behavioral variables in rodents (e.g. Vadacz, Cobor & Lajtha, 1992). Our study found different effects of temporal factors on the development and the appearance of grooming. The results confirm the findings of Bindra and Spinner (1958) and Shtemberg (1982) in that the number of open field grooming episodes increased after the third minute of the test; this increase was especially prominent in brown rats (NR). Unfortunately, the cited works contain no information on the temporal characteristics of grooming episodes at various experimental stages. The present experimental results demonstrated that grooming episodes of different durations displayed different features across the course of the test. There was an increase in the number and proportion of prolonged episodes (over 21 s in duration) across the test in different rat groups. Similar changes in the temporal characteristics were also found in the course of our other test, with the last segments of the test characterized by more frequent grooming, as well as by longer latencies and durations of grooming episodes (Semiokhina & Pleskacheva, 1989). Obviously, the temporal characteristics of grooming may characterize an animal's state in the course of the experiment.

Long-duration grooming reactions are presumably caused by inhibitory processes, since they are accompanied by a decrease in the animals' motor activity. These episodes are probably of the type which Delius (1970) proposed to be associated with a brain arousal-inhibitory mechanism. The long latency for their occurrence may be due to fear.

New and frightening stimuli in the open field evoke other reactions like escape or freezing more frequently during the initial phase of a test, and these reactions compete with grooming and suppress it (Doyle & Yole, 1959; Fentress, 1973; Van Erp, Kruk, Meelis & Willekens Bramer, 1994). It is clear that short-duration episodes were not connected with the specific stage of the test and/or the decrease in locomotion. The number of grooming episodes positively correlated with rearing postures and number of crossed squares (shown in our data from the KM strain, see also Satinder, 1968; Titov & Kamensky, 1980).

It seems possible that prolonged grooming episodes have other bases and biological functions than do short-duration grooming reactions. This assumption is confirmed by recent data on a possible difference in the neurochemical basis of the factors that initiate and prolong self-grooming. Oxytocin is apparently involved in the initiation of self-grooming in rats, whereas ACTH and alpha-melanocyte-stimulating hormone prolong grooming initiated by other means, e.g. a novel environment (Van Erp, Kruk, Semple & Verbeet, 1993). Moreover, there are findings of different effects of intraventricular administrations on the temporal characteristics of grooming: ACTH prolonged grooming, without changing the number of episodes, whereas beta-endorphins increased the number of episodes (Gispen & Isaacson, 1981). Obviously, a more detailed investigation of the temporal characteristics of natural grooming, as well as the effects of various drugs on grooming, should make it possible elucidate the mechanisms of grooming, especially the possibility that it is a protective response in stressful situations.

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