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Food neophobia in wild and laboratory rats (multi-strain comparison)

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ABSTRACT

Although empirical studies comparing neophobia in wild and laboratory rats have been conducted in the past, a few decades have passed since most of them were completed. This is a substantial period of time in the case of fast-breeding animals such as rats. Equally important are the inconsistencies in research findings with respect to comparisons between wild and laboratory rats, and within domesticated strains. As well as having the aim of updating knowledge of neophobia among different types of rats, the present experiment was also an attempt to isolate a specific fear of a new food from a general fear of a novel object.

The procedure was that rats accustomed to one type of food served in a specific location and in a familiar container were given a different type of food. Test trials were preceded by food deprivation. The following variables were measured: feeding latency, the pace of eating, the number of approaches to the container, and the number of times food was sampled in each trial. The amount of food consumed in each trial was weighed and also taken into account. Grooming time served as the measure of stress among the rats in the experiment.

The results of the experiment did not confirm the assertion of some authors that wild rats avoid eating unfamiliar foods. All groups demonstrated only a temporary decrease in the amount of food consumed, the magnitude of which was similar in all strains. No evidence of particularly low neophobia in albino rats was found. However, the behavioral symptoms indicated higher levels of stress in wild rats compared to the other groups.

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1. Introduction

Caution is a natural response of rats to unfamiliar objects (Hebb, 1946). This is also the case when they are confronted with physical changes to the environment and variations in feeding routines (Barnett, 1963, 2009). It is claimed that many rats will not eat unfamiliar food. This strategy may persist for several days at a time, and may be more pronounced in an unfamiliar environment (Burritt and Provenza, 1997; Chapple et al., 1987; Cheney and Miller, 1997). When encountering a novel food, an animal does not know whether it is edible or not. It has to suppress its initial neophobia, and then evaluate the consequences of eating the new food (Barnett, 1963, 2009). The term coined for the avoidance of unfamiliar food is food neophobia (Barnett, 1958, 1963, 2009). It is present in many species (Addessi et al., 2004; Bryan, 1987; Inglis et al., 1996; Kronenberger and Médioni, 1985; Launchbaugh et al., 1997), including rats (Barnett, 1963, 2009; Caroll et al., 1975; Cowan, 1977; Inglis et al., 1996; Mitchell, 1976). The need to distinguish between edible and inedible food is particularly relevant to omnivores, which face the so-called generalist's dilemma (Rozin, 2000).

Rats respond to changes of the location where food appears, changes of the container in which the food is provided, as well as changes of the feed itself (Barnett, 1963, 2009; Caroll et al., 1975; Cowan, 1977; Inglis et al., 1996; Mitchell, 1976). Their response to these novel conditions results from the interaction of behaviors motivated by curiosity about the novel object's potential value and fear of its possible toxicity. This behavior is typified by initial avoidance of a new food, followed by gradual sampling in regular time intervals (Barnett, 1963, 2009). If the new food does not become associated with adverse body symptoms, its intake increases (Barnett, 1963, 2009). The hungrier the rat, the quicker it starts to eat unfamiliar food (Barnett, 1963, 2009). Rats develop an aversion to foods which cause adverse effects within a couple to a dozen hours (Hankins et al., 1973; Revusky and Bedarf, 1967). It has been hypothesized that rats display caution when first encountering new food because they have no gag reflex and, as a result, are unable to eliminate toxic substances from the stomach (Barnett, 1956). It is often claimed that food neophobia is an innate phenomenon (Moron and Gallo, 2007) and that it persists in genetically

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wild rats, even those reared in a laboratory (Barnett, 1958; Galef and Whiskin, 2003). However, some researchers have suggested that food neophobia may be primarily a response learned in the process of socialization (Barnett, 1956, 2005; Taylor and Thomas, 1989).

The container in which new food is served appears to play an important part. Some researchers have asserted that rats prefer food served in a familiar container (Mitchell et al., 2005) and that fear of a new container is significantly stronger than food neophobia itself (Inglis et al., 1996).

There is a lot of empirical data on anatomical and behavioral differences between wild and laboratory rats (Barnett et al., 1979; Blanchard et al., 1986; Huck and Price, 1975; Himmler et al., 2013, 2015; Lockard, 1968; Price, 1999; Stryjek et al., 2012a,b, 2013). It has also been suggested that laboratory rats are characterized by lower neophobia than their wild counterparts (Barnett, 1958; Calhoun, 1963; Cowan, 1977; Mitchell, 1976; Tanaś and Pisula, 2011). The underlying cause for the development of this trait may be the lack of predatory pressure in laboratory conditions, combined with low environment variability. The absence of natural pressures may have significantly diminished the natural constraints of stimulus-seeking behavior in laboratory rats, an activity which plays an important role in adapting to a natural environment (Pisula, 2007). In addition, the changeability of wild rat's habitats may have led to the development of avoidance responses of varying intensity towards a number of environmental changes. Some researchers have suggested that attempts by humans to eradicate rats in their environment may have contributed to the development of food neophobia (Barnett, 1956, 2005; Inglis et al., 1996; Taylor and Thomas, 1989). Rat species not subjected to population suppression through the use of rat poison due to their human-independent diet do not demonstrate neophobic responses to novel foods (Barnett, 1956, 2005; Cowan, 1977; Inglis et al., 1996). Furthermore, food neophobia seems to be absent in Norway rats inhabiting landfills, given their constantly changing environment and the ubiquity of novelty (Barnett, 1963, 2009; Boice, 1971). Absence of food neophobia has also been reported in a group of *Rattus norvegicus* which lived for over a century on an island isolated from human activity (Taylor and Thomas, 1989). Another possibility is that the ancestors of laboratory rats were captured precisely because they exhibited lower neophobia (Mitchell, 1976). These rats may have been less cautious when they encountered bait placed in the trap.

Even though several studies comparing neophobia levels in wild and laboratory rats were conducted in the past, many date back to the 1950s (Barnett, 1958; Cowan, 1977; Calhoun, 1963; Mitchell, 1976). During the long period of laboratory breeding since that time, more changes may have developed in the behavior of laboratory rats. There are also significant inconsistencies in conclusions drawn from comparisons of different lines. The frequently referenced paper by (Barnett, 1958) claimed that laboratory rats demonstrated no food neophobia, in contrast to highly food-neophobic wild rats. Other researchers have suggested that both wild and laboratory rats are food-neophobic (Mitchell et al., 1973), the only difference being that in wild rats food neophobia is stronger (Mitchell, 1976). Mitchell suggested that these differences may have resulted from distinct causes of behavior. He claims that wild rats are afraid of a novel food, while laboratory rats are curious about it. This statement is in accordance with our earlier findings (Pisula et al., 2012), which showed a clear positive response towards novel objects in laboratory rats, but not in wild subjects. This categorization, however, is not consistent with the conclusions of other researchers (Rozin, 2000), who have pointed out that omnivores demonstrate both tendencies at the same time. A more plausible explanation is that fear, as a response to novel food, dominates in wild rats that have adapted to a threatening environment. Furthermore, (Mitchell, 1976) claimed that wild rats were much more averse to eating from an unfamiliar food container than hooded laboratory rats, with albino rats demonstrating the lowest aversion. In his studies, all strains exhibited increased feeding latency when an unfamiliar food container was introduced, but only albino rats showed a decrease in food intake. Still, all rats initially demonstrated aversion to the new container, which means that differences are in the intensity of neophobia rather than in its presence or absence.

It therefore seems necessary to conduct further experiments aimed at systematising knowledge on food neophobia. It is particularly important to control the potential effect of a novel container introduced with novel food, to test multiple strains of laboratory rats (both pigmented and albino strains), and to update the information obtained in studies conducted many decades ago. Furthermore, the experimental procedure described below was designed to reduce the amount of stress experienced by tested animals through shorter testing times and limited length of food deprivation. The novelty of the testing environment was reduced by conducting the experiment in a cage as identical as possible to the ones the rats lived in. Additionally, simple and non-invasive tests associated with the observation of food neophobia in animals may measure their levels of fear. This would be particularly useful in the context of growing interest in emotional processes in animals.

2. Methods

2.1. Ethics statement

All procedures described in this paper were approved by the 4th Local Ethics Commission on Animal Experimentation, Warsaw, Poland. All rats prior to the experiment were cared for in accordance with the Regulation of the Polish Minister of Agriculture and Rural Development of 10 March 2006 on laboratory animal care.

2.2. Animals

The sample consisted of 51 adult rats *Rattus norvegicus*. Experimental groups included 12 Long Evans laboratory rats (6 females and 6 males), 14 Brown Norway laboratory rats (6 females and 8 males), 14 Sprague-Dawley laboratory rats (7 females and 7 males) and 11 wild WWCPS rats (6 females and 5 males).

The WWCPS (Warsaw Wild Captive Pisula Stryjek) rats were derived in 2006 from a sample originating from 5 independent colonies of feral rats (Stryjek and Pisula, 2008). The experiment used the third generation (F3) of laboratory-reared WWCPS wild rats. In order to prevent the development of domestication features in the breeding colony, we systematically include wild rats (freshly caught in a variety of locations) in the breeding scheme. As a result, we have fourth generation laboratory-reared animals at most.

The Brown Norway and Sprague-Dawley rats were sourced from the Mossakowski Medical Research Centre at the Polish Academy of Sciences, while the Wistar rats were taken from the Experimental Medicine Centre at the Medical University of Bialystok, Poland.

Prior to the experiment all rats were housed in groups of 3–5 in Eurostandard type IV cages with ad libitum access to water and standard laboratory fodder. The day/night cycle was set at 12/12 h.

Prior to testing, the rats were weighed and the results were recorded in grams. Females were lighter than males in three lines (WWCPS – $M_f = 214(SD = 23.3)$, $M_m = 272(SD = 20.5)$, $t(9) = 4.308$, $p < 0.01$; Sprague-Dawley – $M_f = 292(SD = 9.5)$, $M_m = 370(SD = 21.7)$, $t(12) = 8.679$, $p < 0.001$; Brown Norway – $M_f = 190(SD = 36.3)$, $M_m = 382(SD = 25.3)$, $t(12) = 11.692$, $p < 0.001$). There were no sex differences in the weights of the Long Evans rats ($M_f = 280(SD = 24.12)$, $M_m = 296(SD = 14.2)$, $p > 0.05$).

There were also weight differences between individual strains (ANOVA – $F(3;47) = 4.512$, $p < 0.01$). However, Tukey's post hoc test showed that the differences were mostly accounted for by Sprague-Dawley rats ($M_{SD} = 331.1$, $SD_{SD} = 43.4$), which were heavier than all other strains ($M_{WWCPS} = 240.4$, $SD_{WWCPS} = 36.7$ $M_{LE} = 288.2$, $SD_{LE} = 20.5$; $M_{BN} = 299.7$, $SD_{BN} = 102.7$).

Further comparisons of body weights within each sex group showed that there were differences among strains in males (ANOVA – $F(3;22) = 40.412$, $p < 0.001$). Post-hoc analysis using Tukey's test showed that male wild rats were of a similar weight to male Long Evans rats ($p > 0.05$), while male Brown Norway rats did not differ significantly in terms of weight from male Sprague-Dawleys ($p > 0.05$). Similar analysis showed differences among strains in females (ANOVA – $F(3;21) = 25.751$). Tukey's post hoc test showed that female wild rats weighed similarly to female Brown Norway rats ($p > 0.05$), while female Long Evans rats did not differ significantly in terms of body mass from female Sprague-Dawley ($p > 0.05$).

2.3. Procedure

2.3.1. Introduction of a novel container

An additional novel container was introduced into a standard laboratory home cage with built-in u-shaped feed hopper. The container was a shallow oval brown ceramic dish (13/9 cm diameter; 2.5 cm in height; 1.5 cm deep) attached to the corner of the cage. It was filled with pulp made from usual rat's feed ground and mixed with water. For 4 days, rats had access to food in the cage's feed hopper, as well as to the food in the new container. Both were replenished daily. The food from the cage's feed hoppers as well as from the novel container were withdrawn 22 h before beginning the experiment. The new container was left empty inside the cage. Access to water remained unlimited.

2.3.2. Habituation trials

Following 22 h of food deprivation, each rat was transferred from its home cage to a new test cage. The test cage was identical to the one in which it had been living. The new container was placed in the same place and filled with the same pulped food. The weight of the food was measured at the end of each trial to determine the amount consumed. After the trial, the rats were individually placed in new clean cages. They were given access to laboratory rat's feed in the feed hopper for two hours. They were then deprived of food for a further 22 h. Each habituation trial lasted for 10 min and was repeated over 3 consecutive days (once per day).

To avoid animals transferring cues about the novel food to each other and to provide the opportunity for uninterrupted eating before the next period of food deprivation, the rats were kept separate during the experiment.

2.3.3. Test trials

The procedure in the test trials was similar to the procedure used in the habituation trials with only one exception. In the new container, rats were given a novel food. The novel food was the pulped food identical to that used in the habituation trials, but with the addition of cinnamon (2 g cinnamon/100 g standard feed). Similarly, after the trial, the rats were individually placed in their cages. They were given access to laboratory rat's feed in the feed hopper for two hours. They were then deprived of food for a further 22 h. Each test trial lasted for 10 min and was repeated over 3 consecutive days (once per day).

The rat's behavior was recorded using a camcorder equipped with an infrared illuminator connected to a computer, allowing for simultaneous recording and real-time observation of the animal's behavior. The camera was placed in front of the cage with a zoom focused on the food container. The rest of the cage was seen in the background of the shot. This set-up allowed us to observe eating behaviors in detail (licking, biting, jaw movements, etc.).

The following variables were measured: latency of eating (the time from beginning of measuring to the start of gnawing), the number of approaches to the container, the number of times food was sampled before eating and total eating duration for each trial. The amount of food consumed in each trial was weighed and also taken into account. As a measure of stress response, the amount of time each rat spent on grooming was assessed (D'Aquila et al., 2000; van Erp et al., 1994; Katz et al., 1981; Komorowska and Pisula, 2003; Thor et al., 1988).

Behaviors were coded from the recorded material using EthoLog 2.2 software (Ottoni, 2000).

3. Results

To enhance the legibility of the results, graphs, and tables, successive habituation trials are presented as $h1$, $h2$, and $h3$, and test trials as $t1$, $t2$, and $t3$, respectively. Novel food was introduced in the first test trial ($t1$).

The data were analyzed using an analysis of variance with repeated measures (ANOVA), with strain (WWCPS, LE, BN or SD) and sex as independent variables and repeated measurements as the dependent variable. Differences were considered significant for p values of ≤ 0.05.

3.1. Amount of food eaten

The amount of food consumed by each rat was calculated using the following formula: Food Amount $= A/B \times 1000\,g$, where A stands for the amount of food eaten during a trial (g) and B represents the rat's body weight (g). The amount of food consumed by each strain in consecutive trials is shown in Fig. 1.

An ANOVA involving two between-subjects factors (four strains × two sexes) and one within-subjects factor (six trials) for the amount of food eaten showed a significant trial difference ($F(3132) = 38.78$, $p \leq 0.001$); and a significant strain by trail interaction ($F(9132) = 3.32$, $p \leq 0.001$). There was no strain × sex × trial interaction effect ($p \geq 0.05$), nor a significant trial by sex interaction ($p \geq 0.05$).

Next, individual trials were compared in pairs using Student's t-test (see Table 1).

The results presented in Table 1 indicate that in the habituation trials the amount of food consumed increased in all groups of laboratory rats, while it remained constant in wild rats. A significant decrease in food consumption was noticed in all groups after the introduction of a novel food ($t1$). The ANOVA yielded no significant differences in the level of decline of food intake in trial $t1$ between individual rat strains ($p > 0.05$). That decline was followed by an increase in the two subsequent trials among wild, Sprague-Dawley and Brown Norway rats. In Long Evans rats, the increase was noted in the third trial following food change ($h3$).

Analysis of variance (ANOVA) was used to compare the amounts of food eaten by individual strains during each experimental trial and yielded differences between the groups in trial $h3$ ($F(3,47) = 3.624$, $p < 0.05$), trial $t1$ ($F(3,47) = 3.372$, $p < 0.05$) and trial $t3$ ($F(3,47) = 2.778$, $p = 0.05$). However, post hoc analysis using the Games–Howell method for multiple comparisons showed that in the second trial ($h2$), Brown Norway rats consumed significantly more food than both wild rats ($\Delta = 10.42 \pm 3.5$; $p < 0.05$) and Sprague-Dawley rats ($\Delta = 8.48 \pm 2.9$; $p < 0.05$). In trials $h3$–$t3$ wild rats ate less than Brown Norway rats ($h3$: $\Delta = 13.2 \pm 3.6$; $t1$: $\Delta = -11.3 \pm 3.8$; $t2$: $\Delta = -11.3 \pm 3.6$; $t3$: $\Delta = -10.8 \pm 3.9$; $p < 0.05$) and less than Sprague-Dawley rats in the $h3$ and $t3$ trials ($h3$: $\Delta = -8.5 \pm 2.8$; $t3$: $\Delta = -12.4 \pm 3.8$; $p < 0.05$).

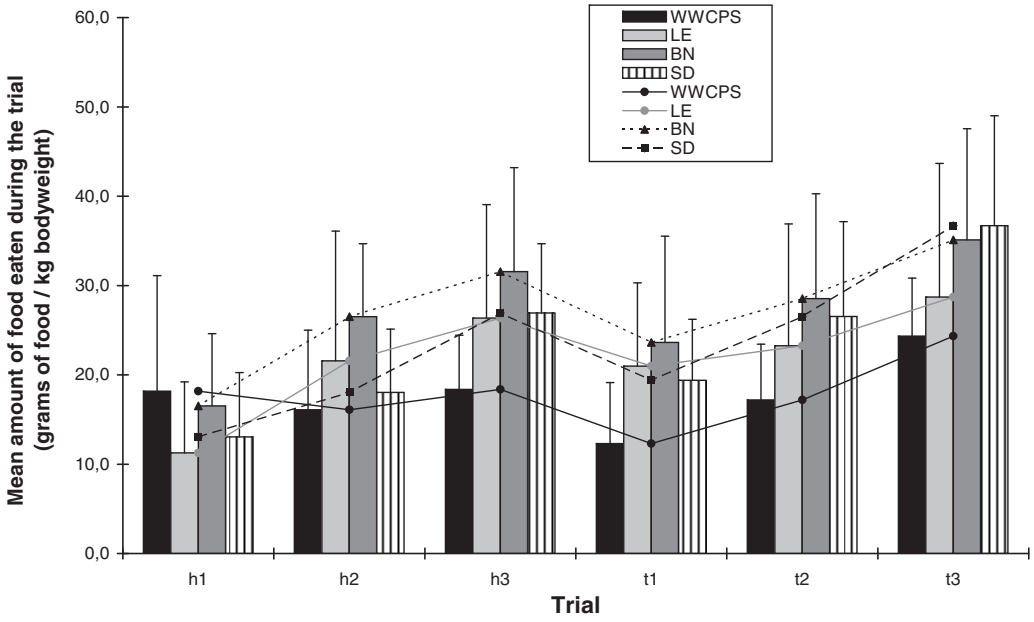

Fig. 1. Mean amount of food eaten in individual trials by each strain.

There was a significant sex by strain interaction ($F(3,43) = 8.23$, $p \leq 0.001$), with Long Evans males eating more ($M = 28.9 \pm 10.0$) than WWCPS and Long Evans females (WWCPS: $M = 15.0$, SD = 3.2; Long Evans: $M = 15.1$, SD = 3.7), $p \leq 0.05$; and Brown Norway females ($M = 33.6$, SD = 10.7) eating more than females from the other stains (Sprague-Dawley: $M = 21.1$, SD = 3.6), $p \leq 0.05$.

3.2. Pace of eating

The pace that each rat ate feed was calculated using the following formula: Food Amount (g)/$C \times 60$ where, C stands for total time of eating during a trial (s).

An ANOVA involving two between-subjects factors (four strain × two sexes) and one within-subjects factor (six trials) for the pace of eating showed a significant trial difference ($F(2,65) = 10.78$, $p \leq 0.001$); but no significant trial by strain interaction ($p \geq 0.05$), nor significant trial by sex interaction ($p \geq 0.05$) or significant strain by sex by trial interaction ($p \geq 0.05$).

According to a Student's t-test there was a significant decline in the pace of eating (g/min) in third habituation trial ($h3$): $t(50) = 2.368$, $p < 0.05$ ($M_{h2} = 9.1$, $SD_{h2} = 6.0$; $M_{h3} = 7.7$, $SD_{h3} = 5.0$) and in $t1$ (after the introduction of the novel food): $t(49) = 5.682$, $p < 0.001$ ($M_{t1} = 5.5$, $SD_{t1} = 4.3$). Finally, there was a significant increase in the pace of eating in $t3$: $t(50) = -2.060$, $p < 0.05$ ($M_{t2} = 5.7$, $SD_{t2} = 2.5$; $M_{t3} = 6.5$, $SD_{t3} = 2.7$).

There was also a significant main effect of sex $F(1,42) = 11.29$, $p < 0.01$, with females eating faster than males (females: $M = 9.5$, SD = 4.7; males: $M = 6.1$, SD = 2.1), but there was no significant strain by sex interaction ($p \geq 0.05$).

3.3. Latency to eat

The latency to eat by each strain in consecutive trials is shown in Figs. 2 and 3.

An ANOVA involving two between-subjects factors (four strain × two sexes) and one within-subjects factor (six trials) for the latency to eat showed a significant trial difference

Table 1
Comparisons of individual experimental trials in terms of amounts of food eaten by each strain (Student's t-test). M – mean value ± standard deviation; t – test value, p – significance, $h1$–$h3$ – habituation trials, $t1$–$t3$ – test trials.

		Compared trials								
		$h1$–$h2$		$h2$–$h3$		$h3$–$t1$		$t1$–$t2$		$t2$–$t3$	
WWCPS	M	18.2 ± 12.9	16.1 ± 8.9	16.1 ± 8.9	18.4 ± 6.1	18.4 ± 6.1	12.3 ± 6.8	12.3 ± 6.8	17.2 ± 6.2	17.2 ± 6.2	24.3 ± 6.5
	t	1.044		−.936		3.069		−3.360		−4.350	
	p	.321		.371		.012		.007		.001	
LE	M	11.3 ± 7.9	21.6 ± 14.5	21.6 ± 14.5	26.3 ± 12.7	26.3 ± 12.7	21.0 ± 9.3	21.0 ± 9.3	23.2 ± 13.6	23.2 ± 13.6	28.7 ± 14.9
	t	−3.188		−2.079		2.427		−1.073		−2.703	
	p	.009		.062		.034		.306		.020	
BN	M	16.5 ± 8.1	26.5 ± 8.2	26.5 ± 8.2	31.6 ± 11.6	31.6 ± 11.6	23.6 ± 11.9	23.6 ± 11.9	28.5 ± 11.7	28.5 ± 11.7	35.1 ± 12.4
	t	−6.026		−2.579		4.674		−2.523		−7.742	
	p	.000		.023		.000		.025		.000	
SD	M	13.05 ± 7.2	18.0 ± 7.1	18.0 ± 7.1	26.9 ± 7.8	26.9 ± 7.9	19.4 ± 6.8	19.4 ± 6.8	26.5 ± 10.6	26.5 ± 10.6	36.7 ± 12.3
	t	−2.282		−7.273		2.999		−3.227		−3.482	
	p	.040		.000		.010		.007		.004	

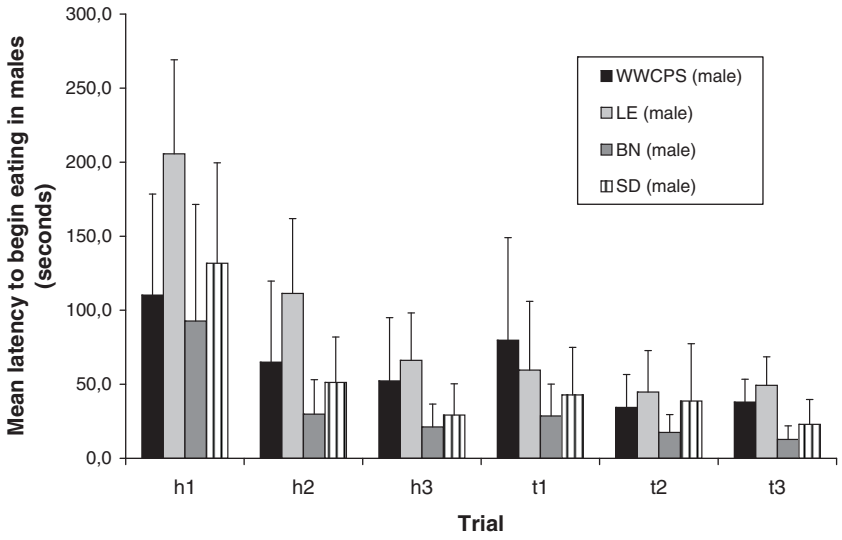

Fig. 2. Mean latency to begin eating in successive trials for each strain of rats, in males.

($F(2,99) = 37.1$, $p \leq 0.001$), a significant trial by stain interaction ($F(7,99) = 2.43$, $p \leq 0.05$) and a significant trail by sex interaction ($F(2,99) = 2.98$, $p \leq 0.05$). There was no strain × sex × trial interaction effect ($p \geq 0.05$).

Next, individual trials were compared using a Student's *t*-test. In all of the study groups there was a statistically significant decrease in eating latency between the first ($h1$) and the third habituation ($h3$) trials: WWCPS rats $t(10) = 2.850$, $p < 0.05$; Long Evans rats $t(11) = 4.669$, $p = 0.001$; Brown Norway rats $t(13) = 4.760$, $p < 0.001$; and Sprague-Dawley rats $t(13) = 3.302$, $p < 0.05$. Following the introduction of the new food, eating latency in all groups of rats stopped decreasing and remained constant until the end of the experiment.

An analysis of the differences in eating latency between rat strains in successive experimental trials was conducted using an ANOVA ($h1$: $F(3,47) = 3.264$, $p < 0.05$; $h2$: $F(3,47) = 8.858$, $p < 0.001$, $h3$: $F(3,47) = 6.182$, $p = 0.001$; $t1$: $F(3,47) = 7.214$, $p < 0.001$; $t2$: $F(3,47) = 7.313$, $p < 0.001$ and $t3$: $F(3,47) = 10.891$, $p < 0.001$). Post hoc analysis using the Games–Howell method for multiple comparisons showed that WWCPS rats demonstrated higher latency in the analyzed behavior than Brown Norway rats in trials $h2$ ($\Delta = 136.73 \pm 42.16$; $p < 0.05$), $t2$ ($\Delta = 48.32 \pm 13.9$; $p < 0.05$) and $t3$ ($\Delta = 26.62 \pm 6.51$; $p < 0.01$). Brown Norway rats demonstrated lower eating latency compared with Long Evans rats in trials $h1$ ($\Delta = -153.86 \pm 42.42$; $p < 0.05$), $h2$ ($\Delta = -103.07 \pm 18.31$; $p < 0.001$), $h3$ ($\Delta = -66.65 \pm 18.66$; $p < 0.05$), $t2$ ($\Delta = -47.31 \pm 10.95$; $p < 0.01$) and $t3$ ($\Delta = -98.15 \pm 6.84$; $p < 0.001$), and lower than Sprague-Dawley rats in trial $h2$ ($\Delta = 34.10 \pm 12.12$; $p < 0.05$). Moreover, eating latency among Sprague-Dawley rats was lower compared with Long Evans rats in trials $h2$ ($\Delta = -68.97 \pm 20.47$; $p < 0.05$) and $t3$ ($\Delta = -24.15 \pm 8.06$; $p < 0.05$). Although the ANOVA found differences between strains in trial $t1$ (following the introduction of the novel food), detailed post hoc analysis did not support this result.

A Student's *t*-test was used to assess the differences between sexes with regard to latency to eat. In males, there was a significant decline in the second ($h2$): $t(25) = 5.235$, $p < 0.001$ ($M_{h1} = 132.6$, $SD_{h1} = 79.3$; $M_{h2} = 61.1$, $SD_{h2} = 48.1$) and third ($h3$): $t(25) = 3.854$, $p < 0.001$ ($M_{h3} = 39.7$, $SD_{h3} = 31.8$) habituation trials. There was no significant change in latency to eat after the introduction of

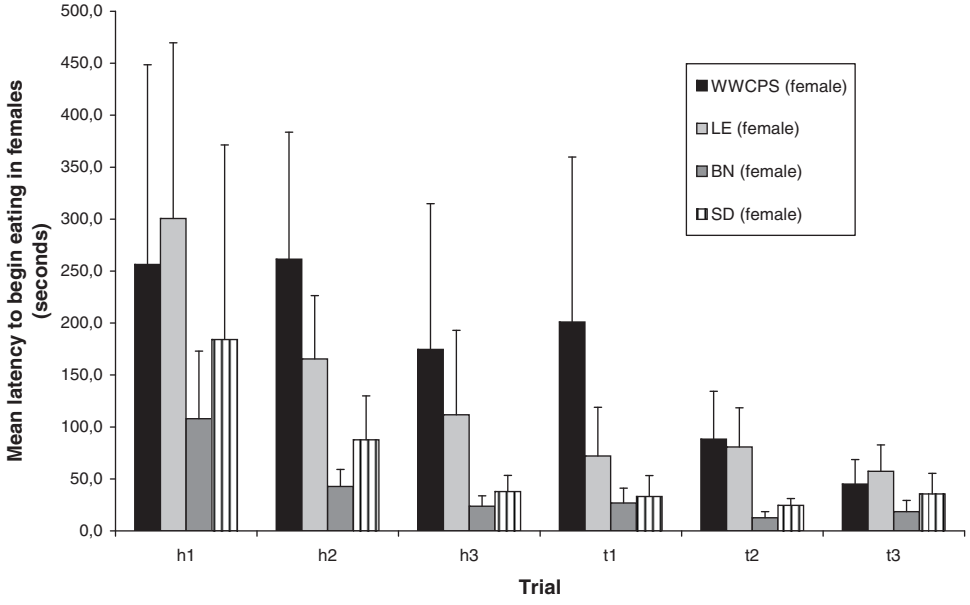

Fig. 3. Mean latency to begin eating in successive trials for each strain of rats, in females.

the novel food ($t1$), $p \geq 0.05$. Following that, there was a significant decline in $t2$: $t(25) = 2.432$, $p < 0.05$ ($M_{t1} = 49.4$, $SD_{t1} = 44.0$; $M_{t2} = 32.8$, $SD_{t2} = 27.5$). In females, there was a significant decline in the second ($h2$): $t(24) = 2.564$, $p < 0.05$ ($M_{h1} = 211.2$, $SD_{h1} = 169.4$; $M_{h2} = 137.3$, $SD_{h2} = 106.7$) and third ($h3$): $t(24) = 3.646$, $p = 0.001$ ($M_{h3} = 85.0$, $SD_{h3} = 96.6$) habituation trials. There was no significant change in latency to eat after the introduction of the novel food ($t1$), nor in $t2$ or $t3$ ($p \geq 0.05$).

According to a Student's t-test, there were differences between sexes in individual trials. Females started eating later than males in $h1$: $t(34) = -2.107$, $p < 0.05$ ($M_f = 211.2$, $SD_f = 169.4$; $M_m = 132.6$, $SD_m = 79.3$), in $h2$: $t(33) = -3.261$, $p < 0.01$ ($M_f = 137.3$, $SD_f = 106.7$; $M_m = 61.2$, $SD_m = 48.1$), and in $h3$: $t(29) = -2.233$, $p < 0.05$ ($M_f = 85.0$, $SD_f = 96.6$; $M_m = 39.7$, $SD_m = 31.8$). There were no differences in $t1$, $t2$, or $t3$ ($p \geq 0.05$).

There was also a significant sex by strain interaction, $F(3,43) = 3.82$, $p \leq 0.05$, with WWCPS females beginning to eat later than Brown Norway and Sprague-Dawley females (WWCPS: $M = 171.1$, SD = 89.3; Brown Norway: $M = 38.8$, SD = 13.3; Sprague-Dawley: $M = 67.1$, SD = 28.8), and later than all males (WWCPS: $M = 63.3$, SD = 33.3; Long Evans: $M = 89.5$, SD = 26.8; Brown Norway: $M = 33.8$, SD = 23.6; Sprague-Dawley: $M = 52.8$, SD = 18.4), $p \leq 0.05$. Long Evans females began to eat later ($M = 131.3$, SD = 48.4) than Brown Norway and Sprague-Dawley males ($p \leq 0.05$) and later than Brown Norway females ($p \leq 0.05$).

3.4. Number of approaches to container before eating

The number of approaches to the food container undertaken by each strain in consecutive trials is shown in Fig. 4.

An ANOVA involving two between-subjects factors (four strain × two sexes) and one within-subjects factor (six trials) for the number of approaches to the container before eating showed a significant trial difference ($F(2,85) = 74.06$, $p \leq 0.001$); and a significant trial by stain interaction ($F(6,85) = 2.95$, $p \leq 0.05$). There was no trial × sex interaction effect ($p \geq 0.05$), nor a significant strain by sex by trial interaction ($p \geq 0.05$).

Individual trials were compared using a Student's t-test. In all study groups there was a statistically significant decrease in the number of approaches to the container between the first ($h1$) and third ($h3$) habituation trials: WWCPS rats $t(10) = 6.088$, $p < 0.001$; Long Evans rats $t(11) = 8.808$, $p < 0.001$; Brown Norway rats $t(13) = 6.245$, $p < 0.001$; and Sprague-Dawley rats $t(13) = 3,383$, $p < 0.01$. At the introduction of the new food ($t1$), the number of approaches stopped decreasing in all study groups. In WWCPS rats, the number of approaches decreased again in the succeeding trial ($t2$) $t(13) = 2.319$, $p < 0.05$. In the remaining groups, the number of approaches remained constant until the end of the experiment.

A detailed analysis of differences in the number of approaches to the food container before feeding between rat strains in individual experimental trials was conducted. An ANOVA revealed differences between strains in the first three trials ($h1$: $F(3,47) = 4.837$, $p < 0.01$; $h2$: $F(3,47) = 11.634$, $p < 0.001$, $h3$: $F(3,47) = 7.460$, $p < 0.001$). Post hoc analysis using the Games–Howell method for multiple comparisons showed that Long Evans rats approached the container more often than Brown Norway rats ($h1$: $\Delta = 4.1 \pm 0.9$, $p = 0.001$; $h2$: $\Delta = 2.4 \pm 0.5$, $p < 0.01$; $h3$: $\Delta = 1.4 \pm 0.4$, $p < 0.05$) and WWCPS rats ($h2$: $\Delta = 2.2 \pm 0.6$, $p < 0.05$; $h3$: $\Delta = 1.6 \pm 0.4$, $p < 0.01$). Additionally, in the second habituation trial ($h2$), Sprague-Dawley rats approached the container more often than WWCPS rats ($\Delta = 1.3 \pm 0.4$, $p < 0.05$) and Brown Norway rats ($\Delta = 1.6 \pm 0.3$, $p < 0.001$).

There was also a main effect of sex $F(1,42) = 11.29$, $p < 0.01$, with females approaching the new container more frequently than males (females: $M = 2.8$, SD = 1.0; males: $M = 2.3$, SD = 0.8), but there was no significant strain by sex interaction ($p \geq 0.05$).

3.5. Number of food samples ingested before feeding

Food sampling consisted of a quick single lick or bite of feed, without any sign of chewing, followed by withdrawing from the container.

The number of food samples ingested by each strain before starting to eat in consecutive trials is shown in Figs. 5 and 6.

An ANOVA involving two between-subjects factors (four strains × two sexes) and one within-subjects factor (six trials) for the number of food samples ingested before feeding showed a significant trial difference ($F(3150) = 17.98$, $p \leq 0.001$) and a significant trial by sex interaction ($F(3150) = 3.63$, $p \leq 0.05$). There was no trial × strain interaction effect ($p \geq 0.05$), nor a significant strain by sex by trial interaction ($p \geq 0.05$).

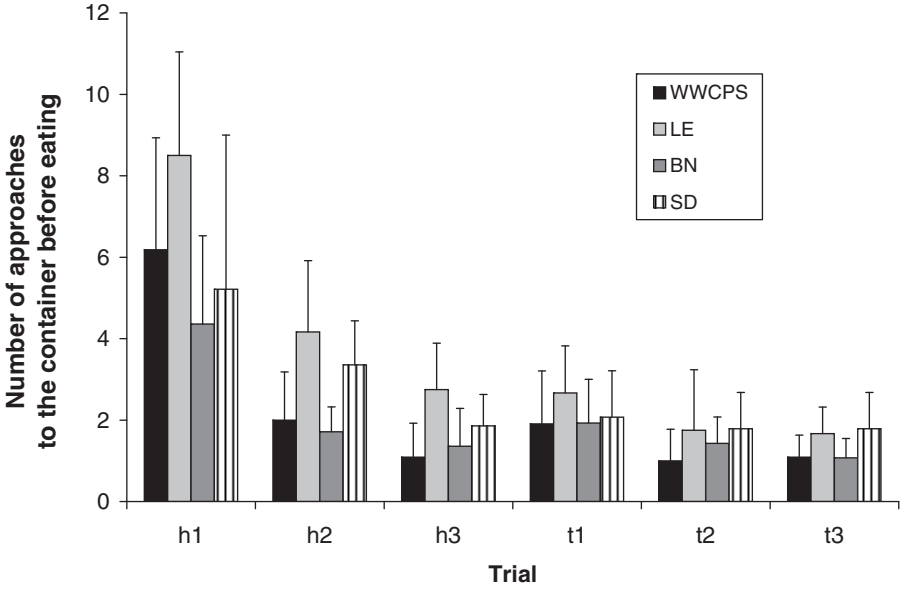

Fig. 4. Mean number of approaches to food container before feeding in each trial for individual strains of rats.

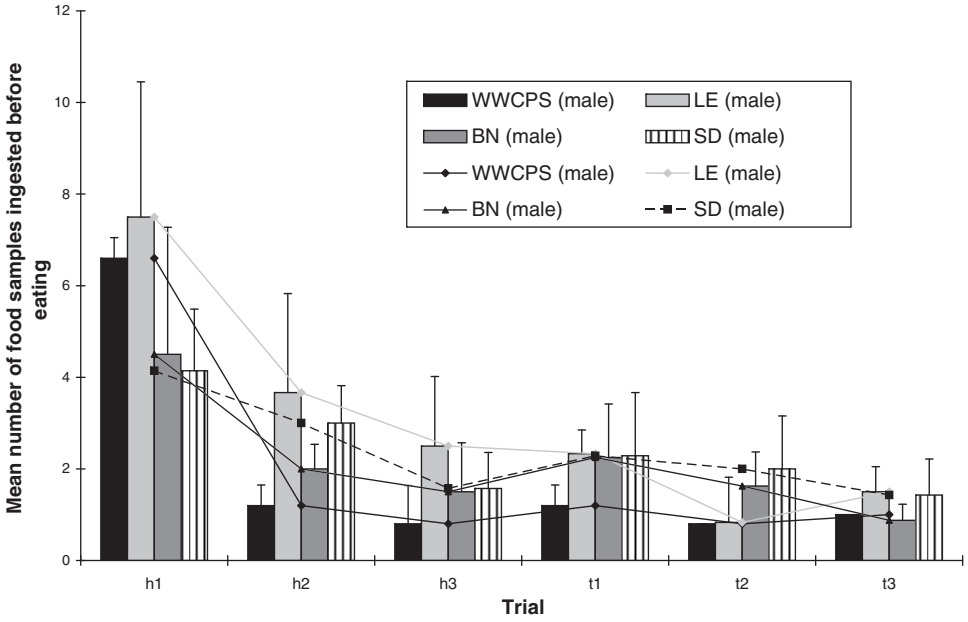

Fig. 5. Mean number of food samples ingested before starting to consume in individual trial, in males.

A Student's *t*-test was used to assess differences between sexes with regard to the number of food samples ingested before feeding. In males, there was a significant decline in the second (*h*2): $t(25) = 3.718$, $p = 0.001$ ($M_{h1} = 1.4$, $SD_{h1} = 0.9$; $M_{h2} = 0.65$, $SD_{h2} = 0.6$) and third (*h*3): $t(25) = 3.934$, $p = 0.001$ ($M_{h3} = 0.1$, $SD_{h3} = 0.4$) habituation trials. There was a significant increase in *t*1 (after the introduction of the novel food): $t(25) = -3.889$, $p = 0.001$ ($M_{t1} = 0.7$, $SD_{t1} = 0.7$). Following that, there was a significant decline in *t*2: $t(25) = 4.835$, $p < 0.001$ ($M_{t2} = 0.04$, $SD_{t2} = 0.2$) and then a significant increase in *t*3: $t(25) = -2.273$, $p < 0.05$ ($M_{t3} = 0.3$, $SD_{t3} = 0.6$). In females, there was a significant decline in the third habituation trial (*h*3): $t(24) = 2.619$, $p < 0.05$ ($M_{h2} = 0.9$, $SD_{h2} = 0.7$; $M_{h3} = 0.5$, $SD_{h3} = 0.5$). There was a significant increase in *t*1 (after the introduction of the novel food): $t(24) = -2.864$, $p < 0.01$ ($M_{t1} = 0.9$, $SD_{t1} = 0.7$). There were no differences in *t*1, *t*2, or *t*3 ($p \geq 0.05$).

According to a Student's *t*-test, there were differences between sexes in individual trials. Females took more food samples than males in *h*3: $t(44) = -2.932$, $p < 0.05$ ($M_f = 0.5$, $SD_f = 0.5$, $M_m = 0.1$, $SD_m = 0.4$) and in *t*2: $t(29) = -5.011$, $p < 0.001$ ($M_f = 0.6$, $SD_f = 0.6$, $M_m = 0.04$, $SD_m = 0.2$). There were no differences in *h*1, *h*2, *t*1, or *t*3 ($p \geq 0.05$).

3.6. Grooming

The time spent on grooming by each individual strain in consecutive trials is presented in Fig. 7.

An ANOVA involving two between-subjects factors (four strains × two sexes) and one within-subjects factor (six trials) for the time spent grooming showed a significant trial difference ($F(4170) = 13.14$, $p \leq 0.001$) and a significant trial by strain

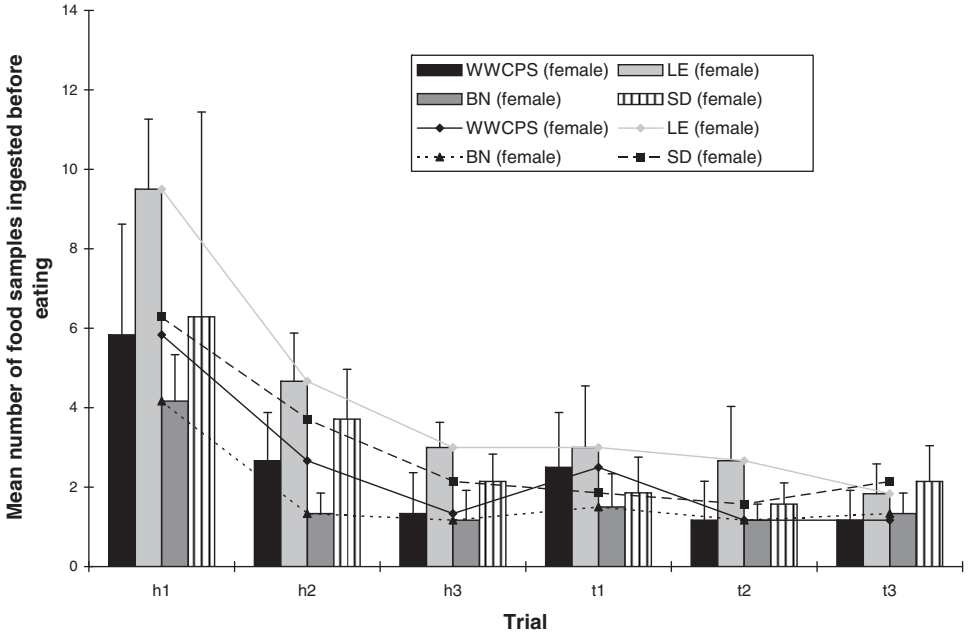

Fig. 6. Mean number of food samples ingested before starting to consume in individual trial, in females.

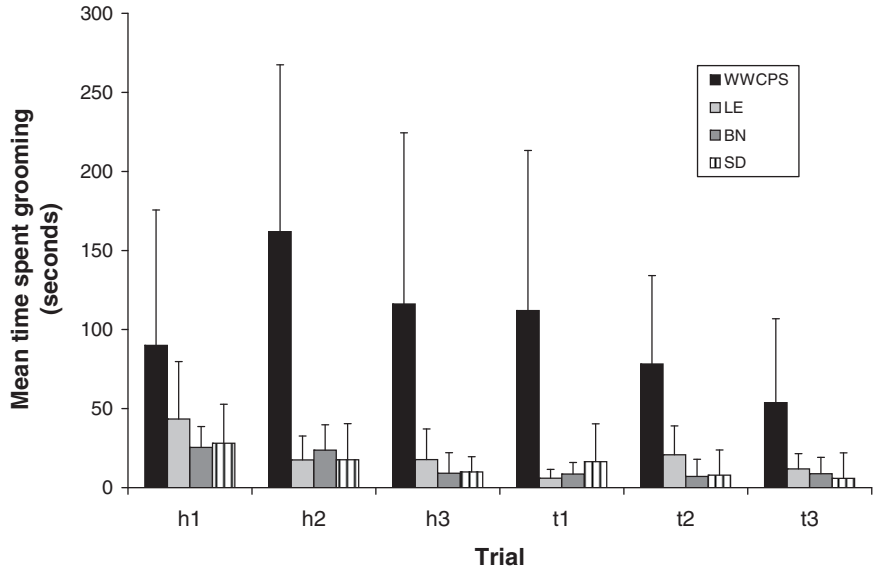

Fig. 7. Mean grooming times in successive trials in the experiment, by group.

interaction ($F(12,170) = 6.75$, $p \leq 0.001$). There was no trial × sex interaction effect ($p \geq 0.05$), nor a significant strain by sex by trial interaction ($p \geq 0.05$).

Individual trials were compared in pairs using a Student's *t*-test. In WWCPS rats there was a noticeable increase in grooming during the first trial ($h1$) of the experiment ($t(10) = -4.287$, $p < 0.01$). In Long Evans rats the time of grooming declined during the first trial ($h1$) of the experiment ($t(11) = 2.484$, $p < 0.05$) and increased in the $t2$ trial ($t(11) = -2.715$, $p < 0.05$). In Brown Norway rats grooming time declined in the second trail ($t(13) = 3.331$, $p < 0.01$) and remained constant to the end of the experiment.

An ANOVA revealed differences between strains in grooming times across all trials ($h1$: $F(3,47) = 5.050$, $p < 0.01$; $h2$: $F(3,47) = 22.097$, $p < 0.001$; $h3$: $F(3,47) = 11.813$, $p < 0.001$; $t1$: $F(3,47) = 12.659$, $p < 0.001$; $t2$: $F(3,47) = 15.729$; $p < 0.001$, $t3$: $F(3,47) = 8.203$, $p < 0.001$). However, post hoc analysis using the Games–Howell method for multiple comparisons showed that only WWCPS rats differed significantly from other strains in some of the trials. WWCPS rats spent more time on grooming than Long Evans rats in trials $h2$ ($\Delta = 144.4 \pm 32.1$, $p < 0.01$), $t1$ ($\Delta = 106.1 \pm 30.5$, $p < 0.05$), and $t2$ ($\Delta = 57.4 \pm 17.7$, $p < 0.05$); more than Brown Norway rats in trials $h2$ ($\Delta = 138.1 \pm 32.1$, $p < 0.01$), $h3$ ($\Delta = 107.0 \pm 32.8$, $p < 0.05$), $t1$ ($\Delta = 103.3 \pm 30.6$, $p < 0.05$), and $t2$ ($\Delta = 71.1 \pm 17.1$, $p < 0.01$); and more than Sprague-Dawley rats in trials $h2$ ($\Delta = 144.3 \pm 32.4$, $p < 0.01$), $h3$ ($\Delta = 106.2 \pm 32.8$, $p < 0.05$), $t1$ ($\Delta = 95.5 \pm 31.2$, $p < 0.05$), and $t2$ ($\Delta = 70.3 \pm 17.4$, $p < 0.01$). There were no significant differences in grooming time between laboratory strains.

There was a significant sex by strain interaction, $F(3,43) = 4.07$, $p \leq 0.05$, with WWCPS females spending more time on grooming than any other group (WWCPS: $M_f = 137.5$, $SD_f = 83.7$; $M_m = 59.4$, $SD_m = 47.4$; Long Evans: $M_f = 21.0$, $SD_f = 5.9$; $M_m = 18.0$, $SD_m = 7.7$; Brown Norway: $M_f = 9.6$, $SD_f = 4.3$; $M_m = 17.0$, $SD_m = 8.3$; Sprague-Dawley: $M_f = 9.1$, $SD_f = 6.8$; $M_m = 19.6$, $SD_m = 19.7$).

4. Discussion

Analysis of the results obtained in the experiment showed that the introduction of a novel food elicited a similar response in all strains. Most importantly, food intake declined similarly in all groups of rats. The first trial with a novel food was also characterized by eating latency becoming stable, a decrease in the pace of eating, a significant increase in the number of times they sampled the food before they started to eat, and an increase in the number of approaches to the food before feeding.

The analysis of differences between groups showed that rats in all groups had achieved comparable values in analyzed variables at the start of the experiment, i.e., in the first habituation trial. However, over the course of the experiment some differences emerged among the groups. Wild rats consumed less food than Brown Norway and Sprague-Dawley strains in some trials, had a longer latency to begin eating than Brown Norway rats and, when novel food was introduced, the number of approaches they made to the food container was higher than those of Long Evans rats. In turn, Brown Norway and Sprague-Dawley rats achieved comparable results with respect to the amount of food consumed, but in the case of latency to begin eating Brown Norway rats showed lower values of that variable compared to Long Evans and Sprague-Dawley rats. However, a characteristic variable that distinguished wild rats from laboratory rats proved to be the duration of grooming. WWCPS female rats demonstrated significantly more of this behavior than any other group. At the same time, there were no significant differences in this respect among laboratory strains.

The comparison of data for male and female rats revealed noticeable sex differences. Brown Norway females ate more than females from the other groups. Moreover, females ate faster and approached the food before feeding more frequently than males. WWCPS and Long Evans females started to eat later than males from other groups.

The present study may suggest that, contrary to the claims of Barnett (Barnett, 1958, 1963, 2009), when presented with novel food, wild rats do not stop eating completely, but only temporarily limit their food intake. Other responses to novel food observed in the experiment included an inhibited decrease in feeding latency and the pace of eating, increases in the number of approaches to the food before eating and a higher number of food samples ingested prior to feeding. Although some differences between groups of rats were found, it was impossible to rank the strains based on levels of food neophobia. We found no evidence in support of Mitchell's (1976) theory that food neophobia is particularly low in albino rats. Contrary to Mitchell's hypothesis, fear of novel food was no lower in albino rats, and their behavior during the experiment was in many respects similar to that of pigmented rats (Brown Norway).

On the other hand, throughout the experiment WWCPS rats demonstrated more behavioral symptoms of stress. Stress may have also affected the assessments of food neophobia levels conducted by other researchers (Barnett, 1958; Calhoun, 1963; Cowan, 1977; Mitchell, 1976). However, in the case of our experiment, it appears to have been unrelated to the appearance of novel food, since there was no increase in grooming following its introduction. A potentially significant difference was that the experimental procedure in the present study ensured a degree of control over the novelty of the container. This factor is associated with rat's response to a novel object, rather than to food novelty alone. In a number of studies, it seems to be the key factor causing inhibition of eating in tested animals (Barnett, 1958; Inglis et al., 1996; Mitchell, 1973).

Another factor explaining the lack of clear differences in food neophobia may be the fact that the WWCPS rats in the experiment were the third laboratory-bred generation and had had no direct contact with animals brought from a natural habitat. Perhaps including freshly captured rats which have faced natural threats, including potentially poisonous food, would provide greater variety in the analyzed behavior (Barnett, 2005; Boice, 1971). This modification, however, would introduce high levels of variability in the experiences among the rats, adding multiple variables which would be impossible to measure and which could potentially affect the results.

By eliminating container novelty and including habituation trials to allow the animals to become accustomed to the experimental conditions, we were able to observe a discrete response to novel food. In addition, analysis of multiple behavior measures provided a wide spectrum of data on the subject of our research. This procedure may have been the reason why our results differ from those obtained by previous researchers (Barnett, 1958; Mitchell, 1976).

The differences between laboratory strains observed in the experiment suggest that the choice of a specific domesticated strain of rats as the experimental animal must be carefully considered. Various strains of laboratory rats may differ in some aspects from one another to a greater extent than they differ from their wild conspecifics (e.g., Himmler et al., 2013, 2015; Stryjek et al., 2012a,b, 2013). Limiting the choice to the most commonly used laboratory strains, without taking into account their specific physiological and behavioral profiles, may lead to false conclusions, either due to specific experimental procedures or unsuitable breeding conditions. This means that conclusions from behavioral analysis performed on a given strain of laboratory rat may only be valid for that particular strain, and any generalization of results to all laboratory rats or the entire species can only be tentative. The same applies to the use of laboratory rats as models of various traits and behaviors.

On the other hand, the conviction held by some researchers, that laboratory rats are pervasively and permanently degenerated to the point that they are unfit for any empirical research (Beach, 1950; Lockard, 1968) appears to be not entirely valid. Considering the high variability among domesticated strains, rats should be selected for empirical research with the utmost care and full awareness of their individual characteristics. This is why it is so important to study differences within the population of laboratory animals and investigate the way they differ from their wild conspecifics. However, empirical data can also be affected by wild rat's inferior adaptation to laboratory conditions, for example due to a particularly high level of stress symptoms. With the high level of fear in this population, a good research practice would be to minimize any stress-inducing aspects of breeding and experimental procedures (Beach, 1950; Stryjek, 2008, 2010; Stryjek and Modlińska, 2013).

5. Conclusions

The results obtained in the present study provide no clear evidence that wild rats demonstrate greater food neophobia than laboratory rats. The patterns of behavior modification and responses to novel food were similar in all the strains tested. However, there were differences between individual strains in the levels of some parameters. Interestingly, these differences were not only observed when wild and domesticated rats were compared, but were also found among laboratory strains.

The only variable that distinctly distinguished wild rats from laboratory rats was the duration of grooming. This symptom of stress was highest in WWCPS females, while there were no significant differences among the laboratory strains. However, this appears to have been unrelated to the appearance of novel food, since there was no increase in grooming following its introduction.

A potentially significant issue was that the experimental procedure in the present study ensured a degree of control over the novelty of the container, which appears to be the main factor causing inhibition of eating in tested animals.

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