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The impact of changeability of enriched environment on exploration in rats

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ABSTRACT

Although the positive effect of environmental enrichment on animals' cognitive capacities is well-known, it remains unclear what role changeability plays in this context. Our study aims to analyse the impact of environmental changeability on the level of exploration and the rate of habituation to novelty.

Prior to the experiment, the animals were housed in three settings: enriched stable conditions, enriched changing conditions and standard conditions. Environmental changeability was introduced by re-arranging objects in the housing pen. A test was conducted to measure the level of exploration in adult individuals.

The study results suggest that rats housed in standard conditions exhibit a higher demand for interactions with the new environment. However, once novelty is introduced, rats from the enriched environments spend more time than their standard counterparts exploring the new objects. No significant differences have been observed in the behaviour of rats from the stable and changeable conditions. It may be concluded, therefore, that in a setting characterised by long-lasting environmental enrichment, the changeability of the environment plays no major role, at least with respect to exploration, general activity and the rate of habituation to novelty. It may be linked to the relatively quick extinguishment of behaviours reinforced by intrinsic reinforcement.

1. Introduction

In laboratory settings, environment can be enriched by providing the animal with a cognitively, physically and socially stimulating living space which enables spontaneous exploration (Baroncelli et al., 2010). Enrichment is often achieved by the introduction of objects (that is, wooden blocks, swings, houses, spinning wheels), as well as more opportunities for social contact (e.g., Bloomsmith et al., 1991; Newberry, 1995), which make the basic settings more complex and more diversified. From an ecological perspective, environmental enrichment results in a larger scope of affordances of the environment available to the animal (for affordances see Gibson, 2014; Rietveld and Kiverstein, 2014). Numerous studies show that animals maintained in enriched conditions perform better in learning tasks (also see e.g.: Gardner et al., 1975; Schrijver et al., 2002), and demonstrate a higher level of exploratory activity and lower anxiety (e.g., Gardner et al., 1975; Genaro and Schmidek, 2001; Pietropaolo et al., 2004). Moreover, environmental complexity increases novelty-seeking behaviour (Fernandez-Teruel et al., 1997) and object exploration (Widman and Rosellini, 1990), while reducing anxiety-like behaviour and increasing activity. What is more, increased environmental complexity evokes changes in behaviour that have been linked to various changes in the brain (e.g., Hebb, 1946; Benaroya-Milshtein et al., 2004; Kolb and Whishaw, 1998; Lewis, 2004; Rosenzweig and Bennett, 1996). Environmental enrichment has a significant impact on the nervous system of both young and mature animals (e.g., Frick and Fernandez, 2003; Camel et al., 1986), may contribute to the reversal of cognitive and emotional impairments (e.g., Dahlqvist et al., 2004; Francis et al., 2002; Jankowsky et al., 2005), and can have a positive effect on animal welfare in captivity (e.g., Abou-Ismail et al., 2010).

Standard environmental enrichment in laboratory conditions often remains constant throughout the entire period of environmental manipulation (e.g. exposure to the enrichment). There are relatively few studies investigating the impact of environmental changeability occurring in those kind of setups. In the natural environment, animals encounter a wide range of environmental stimuli that may change in a variety of ways (eg. Wasserman et al., 2004). This changeability may have a significant impact on their behaviour and may influence different groups of animals to varying degrees. For instance, domesticated animals bred in a stable laboratory or farm environment may react to environmental changeability in a different way than their wild counterparts. The changeability of the living environment may also affect the development of based on previous experience animal expectations for the surroundings to be less or more predictive and controllable. Since the motivation to control environmental events seem to be an important element of animal and human behaviour regulation, the

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generalized experience of unpredictability vs stability may have profound effect on animal cognitive development in general (Bassett and Buchanan-Smith, 2007; Chorpita and Barlow, 1998; Cramer et al., 1997; Job, 2006).

Introducing frequent changes to an animal's environment forces the animal to explore and to continually update internal representations of its environment (Leonard and McNaughton, 1990; Poucet and Benhamou, 1997). The changes affect not only the structure of the environment and the location of objects, but also the location of food sources and the type of food. The unpredictability of changes and the lack of control over the surroundings trigger a stress response (LaDage, 2015). One way of dealing with the constant presence of stimuli resulting from such changes and their unpredictability may involve moderation of the emotional reaction to novelty. This hypothesis is confirmed by the data obtained in a study where rats kept in standard conditions were exposed to changes in experimental setup in every trial (Pisula et al., 2006). However, in individuals with a high level of emotional reactivity, constant change may result in the worsening of cognitive functions (Benus et al., 1987). On the other hand, as Staddon (2016, p. 204) states, "states of nature that make no difference, […], will not usually be differentiated — will not produce different states of the animal." It may follow from that constant changes which are associated with neither reward nor punishment result in decreased sensitivity to the novelty encountered in the environment and do not trigger an emotional response. Even if we assume that constant change is an intrinsic reinforcement leading to increased exploration, intrinsically reinforced, unlearned behaviours such as curiosity habituate both after the introduction of novelty and across consecutive introductions of novelty (Tarou and Bashaw, 2007).

Although the positive effect of environmental enrichment on an animal's cognitive capacities is well-known and widely documented, it remains unclear what precise role environmental changeability plays in the context of environmental enrichment. The current state of knowledge fails to suggest a clear direction of the relationship between such stimuli and later behaviour. Our study aims to analyse the impact of environmental changeability on the level of exploration in rats and the rate of habituation to novelty in the environment explored. The changeability of the enriched environment was ensured in our experiment by changing the arrangement of the objects in the pen on a daily basis.

2. Materials and methods

2.1. Subjects

The sample was comprised of 29 male rats of the Lister Hooded stock (11 rats for enriched stable conditions group; 10 rats for enriched changeable conditions group; and 8 rats for standard condition). The animals were sourced from Charles River, Germany, via AnimaLab Sp. z o.o., Poland. The rats were housed in the vivarium of the Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland.

2.2. Housing conditions

Prior to the experiments, the rats were kept in three different housing setups. The first group was reared in an enriched stable environment. The second group – in an enriched, but constantly changing environment. The third group was reared under standard conditions (control group). The rats were randomly assigned to one of the three sets of conditions. All rats were housed in groups consisting of 4–6 individuals. The groups' configuration was unchanged during the study, so as to maintain a stable social environment for all animals (see Tanaś and Pisula, 2011; Pisula et al., 1992).

2.2.1. Enriched stable conditions (ESC)

The housing area was a pen with combined dimensions of approx. 2000 mm × 1000 mm × 1000 mm, which enabled the rats to move freely in three dimensions. The pen was covered with wire mesh placed on top of a wooden frame. The floor was covered with dust-free softwood granules "Tierwohl Super©". The pen was equipped with objects that enriched the housing environment: a wooden box with two entrances, which provided shelter and a nesting site; two wooden tunnels; three pillar-shaped wooden blocks; a spool of hemp twine suspended on a metal chain; a horizontal wooden bar placed on two posts connected to a ramp covered with wire mesh; a mirror; and a wooden wall covered with a metal net. In addition, inside the pen an open standard breeding cage (Tecniplast© Eurostandard Type IV) was placed. It was fitted with three dispensers (two with water, one with fodder) inside the cage. The purpose of placing on open cage inside the pen was to habituate the animals to the cage and its interior to avoid the novelty effect which would have been induced by putting the animals in that cage before the experiment. The scheme of the housing pen is shown in Fig. 1. The animals were fed standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland), which was put in two places (inside the cage and in a metal bowl outside the cage). The area was also fitted with additional water dispensers.

Fig. 1. Housing pen with objects used for environmental enrichment.

2.2.2. Enriched changing conditions (ECC)

The housing conditions were the same as described in Enriched stable conditions (ESC). However, in this setup, the objects inside the pen were placed in different configurations and locations every day. Similarly to ESC conditions, the animals were fed standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland), which was put in two places (inside the cage and in a metal bowl outside the cage, but the metal bowl was shifted daily). Additional foods were given to the rats to ensure food changeability including: oat flakes, rice flakes, corn flakes, apples and carrots. These types of food were provided individually or in different combinations; they were also sometimes mixed with herbs (dried parsley, coriander, basil, dill). Additionally, the location and type of food was changed daily. We decided not to introduce new objects, which can increase the variability of the environment during the rearing phase, because new objects could have different properties

(e.g., higher potential for motor activities) than objects from the stable environment. It would add another variable in the study and prevented the comparison of the impact of both environments on rats.

2.2.3. Standard conditions (SC)

The rats were housed in groups of 4 in Tecniplast© Eurostandard Type IV cages (610 mm × 435 mm × 215 mm) with dust-free softwood granules Tierwohl Super© as bedding and with ad libitum access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland).

In each of the housing setups, the day/night cycle was set at 12/12 h, with the lights-on at 8 A M; the temperature was maintained at a constant 21–23 °C. The pen arena was lit with fluorescent lamps at 75–100 lx (depending on the location). The cages and pens were cleaned once a week, on the same day and at a fixed time. Fodder and water were replenished daily in all housing setups. All rats kept in our laboratory were housed, bred and taken care of in accordance with the Regulation of the Polish Minister of Agriculture and Rural Development of 14 December 2016 on laboratory animal care; the experimental procedures were approved by the 1 st Local Ethics Committee on Animal Experimentation in Warsaw, Poland.

The animals' behaviour inside the pens was monitored by means of a video camera, and the changes of food and arrangement of the objects were recorded in photos and reports.

2.3. Procedure

Prior to the experiment, at 23 PND, the animals were put in the housing pen. They were kept in one of the experimental setups for a period of three months. The rats kept in standard conditions were put in cages after the end of the weaning stage (at 23 PND), and remained there until the onset of the experiment.

The aim of the exploration test was to compare the process of investigating a new environment, the rate of habituation to it, and the reaction to the introduction of a novelty of low intensity into a well-known context. The apparatus and measurement methods were similar to those used in our previous studies (Pisula, 2003, 2004; Pisula and Siegel, 2005; Pisula et al., 2006; Tanaś and Pisula, 2011; Pisula et al., 2012). The reason for using this apparatus in the present study was that, contrary to most other tests (eg. Open Field Test, Hole Board etc.), it enables a lateral view. This way of observing the animal allows for a detailed observation of its behaviour, which is crucial for detecting emotional reactivity in animals (e.g. grooming).

The experimental chamber (Fig. 2) was a box measuring 800 mm × 600 mm × 800 mm. The chamber was divided into three zones: A, B, and C by two walls running perpendicularly to its longer side. The front of the chamber was a transparent wall which could be lifted to obtain full access to the experimental arena. The wooden division walls between the zones had triangular entrances (120 mm × 140 mm) at the bottom, which enabled free movement between the chamber parts. The entire chamber was covered with a layer of washable varnish. There were two tunnels (200 mm × 120 mm × 80 mm) in zones B and C made of hardwood covered with washable paint. In contrast to the most frequently used two-dimensional experimental settings (eg. Open Field Test, Hole Board etc.), where animals explore flat surfaces, these tunnels provide a complex three-dimensional environment. The animal has the possibility not only of exploring the surface of the experimental arena, but also of climbing and going into the tunnels. The central zone (A) was left empty – there was a hole curved in the back wall of the chamber which served as an entrance for animals going from the transporter into the chamber.

At the start of each trial, a small cylindrical cage (the 'starting box' – 60 mm in diameter with doors 120 mm high and 100 mm wide) with the tested animal inside was placed by the entrance to zone A. The entrance door was then opened and it was left open until the end of the test. The animal was free to stay in the starting box or leave it to explore

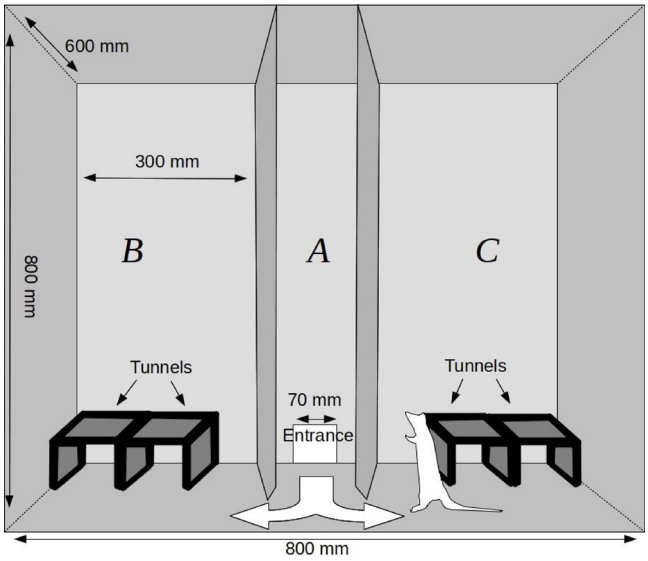

Fig. 2. Experimental chamber used for investigating exploratory behaviours. A - the central zone of the experimental chamber with an entrance to the apparatus; B - the left zone of the experimental chamber (no changes throughout the experiment); C - the right zone of the experimental chamber (novelty in the form of additional tunnels was introduced in this zone - see Fig. 3). The curved arrows at the bottom of the figure show the direction in which rats can move between the zones through the passages cut in the inside walls.

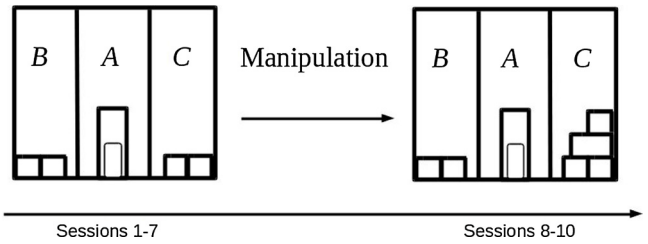

Fig. 3. Arrangement of objects in the experimental chamber during the experiment.

the chamber. The first seven trials were habituation trials during which the apparatus was arranged in the same way (Fig. 3). The introduction of novelty (i.e. the addition of new two tunnels on top of the old ones in zone C) took place between trials 7 and 8. The three subsequent trials were conducted with the chamber in this new arrangement (Fig. 3). Each trial was 7 min long and was conducted for each animal once a day.

A video camera was placed approximately 1.5 m away from the transparent front wall of the experimental chamber. The camera was set in the night-shot mode to enable filming in the dark. Behaviours observed were coded on the basis of the recorded material using the BORIS event logging software (Friard and Gamba, 2016). This program makes it possible to define particular behaviours and to score the time and frequency of selected behaviours. In this study, we scored selected behaviours occurring during the entire experimental session. As a result, the exact time of individual bouts of behaviours, their frequency and, consequently, the total time spent engaging in a given behaviour were assigned specific scores. The behaviours analysed comprised the following: latency to leave the starting box; amount of time spent in the starting box; total time spent in the unchanged zone of the chamber; total time spent in the changed zone of the chamber; time spent on contact with the tunnels in the unchanged zone of the chamber; and total time spent on contact with the tunnels in the changed zone of the chamber. 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).

2.4. Data presentation and statistical analyses

To enhance the legibility of the results, graphs, and tables, two phases were marked out from among all habituation trials: phase H1/H2, which involved the initial measurement of behaviour, that is, the animals' behaviour at the start of the experiment; and phase H6/H7, which measured the effect of habituation to the experimental conditions. Subsequently, all test trials were divided into two distinct phases: T1, when novelty was introduced (i.e. the additional tunnels in zone C); and phase T2/T3, which reflected the level of rats' exploratory behaviour during trials conducted after the introduction of novelty (measuring the level of habituation to change).

The data was analysed using a General Linear Model procedure (GLM), with the housing setups (ESC, ECC and SC) as the between-subject factors, and repeated measurements (H1/H2, H6/H7, T1, T2/T3) as the within-subject factor. Differences were considered significant for p values of ≤ 0.05.

3. Results

3.1. Time spent in zone A (central)

The amount of time spent in zone A (the central zone of the experimental chamber) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(1,28) = 3.677$; $p = 0.004$; $eta^2 = 0.306$), and a significant main factor effect for the phase (Wilks' Lambda - $F(1,27) = 81.985$, $p \leq 0.001$; $eta^2 = 0.908$).

Analysis of variance (ANOVA) was used to analyse the differences in time spent in zone A by individuals from the three housing setups during each phase; it yielded differences between the groups only in phase H1/H2 ($F(2,29) = 10.160$, $p = 0.001$; $eta^2 = 0.429$) - Fig. 4. Post hoc analysis using the Tukey HSD test showed that in the habituation phase H1/H2, the ECC rats spent more time in the central zone than the SC rats ($p \leq 0.001$; $M_{ECC} = 146.2$, $SD_{ECC} = 23.2$; $M_{SC} = 106.6$, $SD_{SC} = 18.7$; Cohen's $d = 1.891$).

A paired samples Student's *t*-test was used to assess the differences in time spent in zone A by animals from the three housing setups between individual phases. In ECC rats, there was a decrease in the amount of time spent in this zone in phase H6/H7 ($t(9) = 2.791$, $p = 0.021$; Cohen's $d = 0.884$), followed by another decrease in the first trial after the introduction of novelty, that is, in phase T1 ($t(9) = 14.325$, $p \leq 0.001$; Cohen's $d = 4.534$), which was subsequently followed by an increase in phase T2/T3 ($t(9) = -2.740$, $p = 0.023$; Cohen's $d = 0.873$). In the ESC group, however, the statistically significant aspect was the decrease in the amount of time spent in the central zone in phase T1 ($t(9) = 6.839$, $p \leq 0.001$; Cohen's $d = 2.160$), followed by an increase in phase T2/T3 ($t(9) = -3.021$, $p = 0.014$, Cohen's $d = 0.966$). In the SC group, a decrease was observed in phase T1 only ($t(9) = 3.826$, $p = 0.004$; Cohen's $d = 1.210$).

3.2. Time spent in zone B (left)

The amount of time spent in zone B (the left zone of the experimental chamber) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(2,27) = 2.848$; $p = 0.018$; $eta^2 = 0.255$), and significant phase differences (Wilks' Lambda; $F(1,27) = 26.516$; $p \leq 0.001$; $eta^2 = 0.761$).

Analysis of variance (ANOVA) was used to compare the amount of time spent in zone B by individuals from the three housing setups during each phase; it yielded differences between the groups only in phase H1/H2 ($F(2,29) = 16.423$, $p \leq 0.001$; $eta^2 = 0.549$) – Fig. 5. Post hoc analysis using the Tukey HSD test showed that in phase H1/H2, standard rats spent more time in the left zone than ESC rats ($p \leq 0.001$; $M_{SC} = 155.5$, $SD_{SC} = 28.2$; $M_{ESC} = 94.3$, $SD_{ESC} = 22.5$; Cohen's $d = 2.242$) and ECC rats ($p = 0.001$; $M_{ECC} = 111.3$, $SD_{ECC} = 22.7$; Cohen's $d = 1.747$). No significant differences between the groups were observed in the other phases.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent in zone B by animals from different housing setups between individual phases. In ECC rats, there was a marked decrease in phase T1 ($t(9) = 2.853$, $p = 0.019$, Cohen's $d = 0.902$), that is, after the introduction of novelty to zone C. In ESC rats, there was a significant increase in phase H6/H7 ($t(9) = -2.602$, $p = 0.029$, Cohen's $d = 0.823$), followed by a decrease in phase T1 ($t(9) = 10.738$, $p \leq 0.001$, Cohen's $d = 3.396$), which was then followed by another increase in T2/T3 ($t(9) = -3.083$, $p = 0.013$, Cohen's $d = 0.975$). In the SC group, there was a marked decrease in the time spent in the left zone in phase H6/H7 ($t(9) = 3.842$, $p = 0.004$, Cohen's $d = 1.215$), as well as in phase T1 ($t(9) = 3.531$, $p = 0.006$, Cohen's $d = 1.117$).

3.3. Time spent in zone C (right)

The amount of time spent in zone C (the right zone of the

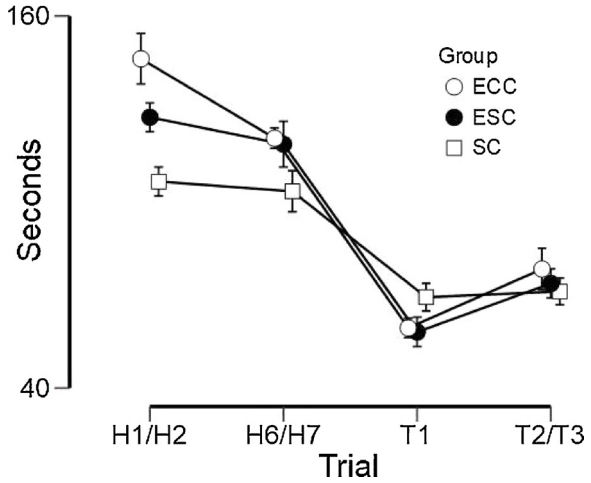

Fig. 4. Time (s) spent by rats in the central zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

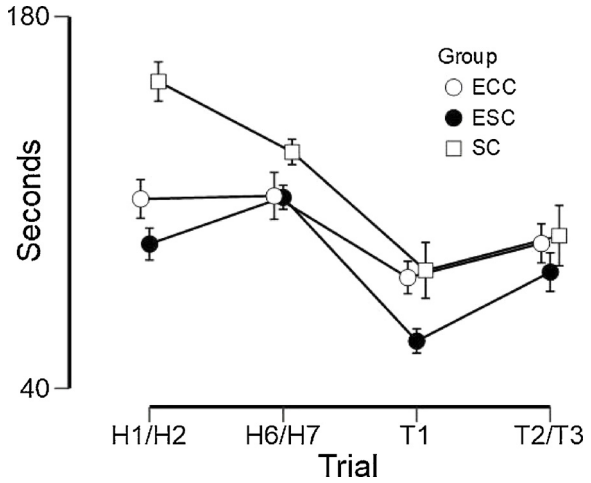

Fig. 5. Time (s) spent by rats in the left zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

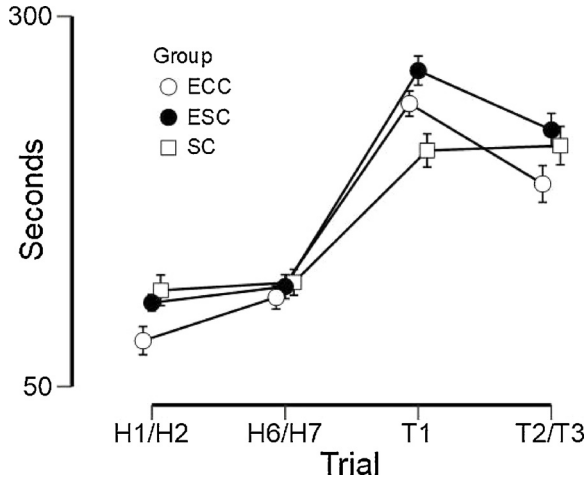

Fig. 6. Time (s) spent by rats in the right zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

experimental chamber) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(1,28) = 2.774$, $p = 0.021$; $eta^2 = 0.250$), and a significant main factor effect for the phase (Wilks' Lambda - $F(1,27) = 100.134$, $p \leq 0.001$; $eta^2 = 0.923$).

Analysis of variance (ANOVA) was used to analyse the time spent in zone C by individuals from the three housing setups during each phase; it yielded differences between the groups in phase H1/H2 ($F(2,29) = 6.079$, $p = 0.007$; $eta^2 = 0.310$), and in phase T1 ($F(2,29) = 6.589$, $p = 0.005$; $eta^2 = 0.328$) – Fig. 6. Post hoc analysis using the Tukey HSD test showed that in the habituation phase H1/H2, ECC rats spent less time in the right zone than their ESC counterparts ($p = 0.045$; $M_{ECC} = 80.9$, $SD_{ECC} = 20.0$; $M_{ESC} = 106.6$, $SD_{ESC} = 24.5$; Cohen's $d = 1.148$) and SC rats ($p = 0.007$; $M_{SC} = 114.9$, $SD_{SC} = 23.4$; Cohen's $d = 1.623$). In phase T1, ESC rats spent more time in zone C than SC rats ($p = 0.003$; $M_{ESC} = 263.4$, $SD_{ESC} = 33.1$; $M_{SC} = 209.4$, $SD_{SC} = 36.1$; Cohen's $d = 1.563$).

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent in zone C by individuals from different housing setups between individual phases. In ECC rats, there was an increase in the time spent in the right zone in phase H6/H7 ($t(9) = -3.103$, $p = 0.013$; Cohen's $d = 0.981$), followed by a marked increase in the first phase after the introduction of novelty, that is, in T1($t(9) = -11.934$, $p \leq 0.001$; Cohen's $d = 3.774$), after which there was a decrease in phase T2/T3 ($t(9) = 3.810$, $p = 0.004$; Cohen's $d = 1.205$). In ESC rats, however, the statistically significant aspect was the increase in the amount of time spent in zone C in T1 ($t(9) = -12.338$, $p \leq 0.001$; Cohen's $d = 3.902$), followed by a decrease in phase T2/T3 ($t(9) = 2.486$, $p = 0.035$; Cohen's $d = 0.786$). In SC rats, there was an increase in phase T1 ($t(9) = -5.580$, $p \leq 0.001$; Cohen's $d = 1.764$).

3.4. Time spent in transporter

The amount of time spent in the transporter, excluding the latency to leave the transporter (that is, the amount of time from the moment the transporter was opened until the rat first entered the experimental apparatus) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(2,27) = 4.225$; $p = 0.002$; $eta^2 = 0.336$), and significant phase differences (Wilks' Lambda; $F(1,27) = 16.735$; $p \leq 0.001$; $eta^2 = 0.668$).

Analysis of variance (ANOVA) was used to analyse time spent in transporter by individuals from the three housing setups during each phase; it yielded differences between the groups only in phase H1/H2 ($F(2,29) = 12.318$, $p \leq 0.001$; $eta^2 = 0.477$). Post hoc analysis using the Tukey HSD test showed that in the first phase, SC rats spent less time in the transporter than ESC rats ($p \leq 0.001$; $M_{SC} = 42.9$, $SD_{SC} = 16.9$; $M_{ESC} = 87.6$, $SD_{ESC} = 26.2$; Cohen's $d = 2.048$) and ECC rats ($p = 0.001$; $M_{ECC} = 84.9$, $SD_{ECC} = 23.5$; Cohen's $d = 2.019$). No differences between the groups were observed in the other phases.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent in the transporter by individuals from different housing setups between individual phases. In ECC rats, there was a significant decrease in the amount of time spent in the transporter in phase H6/H7 ($t(9) = 2.609$, $p = 0.028$; Cohen's $d = 0.825$). In SC rats, however, an increase was observed in phase H6/H7 ($t(9) = -3.628$, $p = 0.006$; Cohen's $d = 1.147$). In the ESC group, there was a marked decrease in T1 ($t(9) = 7.801$, $p \leq 0.001$; Cohen's $d = 2.467$), that is, after novelty was introduced in zone C.

3.5. Time spent on contact with tunnels in Zone B (left)

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(2,27) = 3.360$; $p = 0.007$; $eta^2 = 0.287$), and significant phase differences (Wilks' Lambda; $F(1,27) = 29.788$; $p \leq 0.001$; $eta^2 = 0.781$).

Analysis of variance (ANOVA) was used to analyse the amount of time spent on contact with the tunnels in zone B by individuals from the three housing setups during each phase; it yielded differences between the groups in phase H1/H2 ($F(2,29) = 21.640$, $p \leq 0.001$; $eta^2 = 0.616$); in phase H6/H7 ($F(2,29) = 9.467$, $p = 0.001$; $eta^2 = 0.412$) – Fig. 7. Post hoc analysis using the Tukey HSD test showed that in phase H1/H2, SC rats spent more time on contact with the tunnels than ESC rats ($p \leq 0.001$; $M_{SC} = 96.4$, $SD_{SC} = 15.3$; $M_{ESC} = 58.3$, $SD_{ESC} = 12.5$) and ECC rats ($p \leq 0.001$; $M_{ECC} = 58.7$, $SD_{ECC} = 16.5$; Cohen's $d = 2.370$). Similarly, in phase H6/H7, SC rats spent more time on contacts with the tunnels than ESC rats ($p = 0.018$; $M_{SC} = 81.4$, $SD_{SC} = 15.1$; $M_{ESC} = 60.2$, $SD_{ESC} = 19.6$; Cohen's $d = 1.187$) and ECC rats ($p = 0.001$; $M_{ECC} = 50.8$, $SD_{ECC} = 12.9$; Cohen's $d = 2.137$). No differences between the groups were observed in T1, that is, after novelty was introduced in zone C.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent by animals from different housing conditions on exploration of the tunnels in zone B between individual phases. In ESC rats, a statistically significant decrease in the exploration time was observed in T1 ($t(9) = 5.225$, $p = 0.001$; Cohen's $d = 1.652$), that is, immediately after the introduction of novelty in zone C. In SC animals, however, there was a marked decrease in phase H6/H7 ($t(9) = 2.519$,

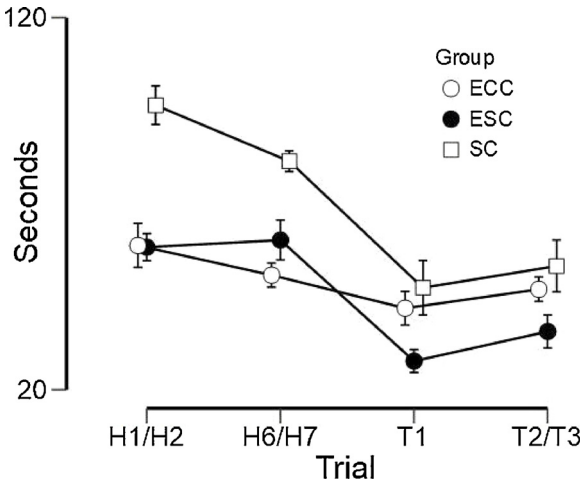

Fig. 7. Time (s) spent by rats on contact with objects in the left zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

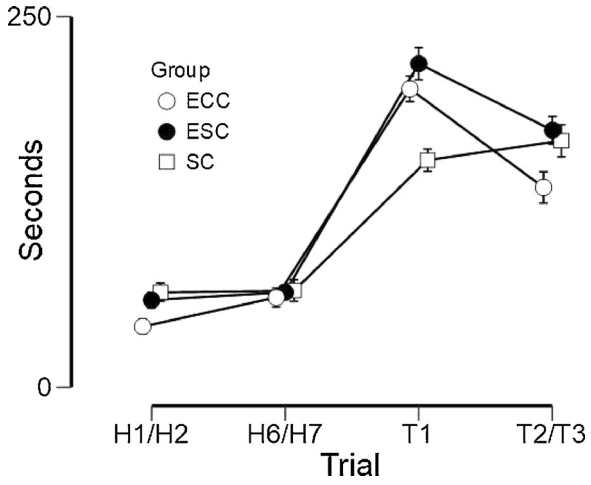

Fig. 8. Time (s) spent by rats on contact with objects in the right zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

p = 0.033; Cohen's d = 0.797), followed by another decrease in exploration time in T1 (t(9) = 4.419, p = 0.002; Cohen's d = 1.397). IN ECC rats, no significant changes were observed between the individual phases of the experiment ($p > 0.05$).

3.6. Time spent on contact with tunnels in Zone C (right)

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; F(2,27) = 4.498; p = 0.001; $eta^2 = 0.351$), and significant phase differences (Wilks' Lambda; F(1,27) = 198.972; $p \leq 0.001$; $eta^2 = 0.960$).

Analysis of variance (ANOVA) was used to compare the amount of time spent on contact with the tunnels in zone C by individuals from the three housing setups during each phase; it yielded differences between the groups in first habituation phase H1/H2 (F(2,29) = 5.883, p = 0.008; $eta^2 = 0.304$), and T1 (F(2,29) = 14.458, $p \leq 0.001$; $eta^2 = 0.517$), that is, immediately after the introduction of novelty – Fig. 8. Post hoc analysis using the Tukey HSD test showed that in the habituation phase H1/H2, SC rats spent more time on contact with the tunnels than ECC rats (p = 0.008; $M_{SC} = 64.0$, $SD_{SC} = 18.4$; $M_{ECC} = 40.9$, $SD_{ECC} = 11.3$; Cohen's d = 1.542), and ESC rats spent more time on contact with the tunnels than their ECC counterparts (p = 0.045; $M_{ESC} = 58.8$, $SD_{ESC} = 16.8$; Cohen's d = 1.187). However, in T1 (after the introduction of novelty), SC rats spent less time on contact with the tunnels than ECC rats (p = 0.002; $M_{SC} = 153.2$, $SD_{SC} = 23.5$; $M_{ECC} = 201.3$, $SD_{ECC} = 26.9$) and ESC rats ($p \leq 0.001$; $M_{ESC} = 218.3$, $SD_{ESC} = 32.9$; Cohen's d = 0.907).

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent by animals from different housing conditions on exploration of the tunnels in zone C between individual phases. In ECC rats, there was an increase in the amount of time spent on contact with the tunnels in phase H6/H7 (t(9) = -3.551, p = 0.006; Cohen's d = 1.123), followed by a sharp decrease in the first phase after the introduction of novelty, that is, T1 (t(9) = -15.002, $p \leq 0.001$; Cohen's d = 4.744), which was subsequently followed by a decrease in phase T2/T3 (t(9) = 4.559, p = 0.001; Cohen's d = 1.442). In ESC rats, on the other hand, the statistically significant aspect was the increase in the frequency of contacts with the tunnels only in T1 (t(9) = -13.437, $p \leq 0.001$; Cohen's d = 4.249), and the subsequent decrease in phase T2/T3 (t(9) = 2.836, p = 0.020; Cohen's d = 0.897). In SC rats, there was an increase in T1 (t(9) = -8.826, $p \leq 0.001$; Cohen's d = 2.791).

Cohen's d was used to estimate the differences in effect sizes for contacts with the tunnels in zone C between the last habituation phase (H6/H7) and the first test phase (T1). The effect size was smaller in the control group (d = 4.57; r = -0.92; $M_{SC\_H7} = 65.2$, $SD_{SC\_H7} = 13.6$; $M_{SC\_T1} = 153.2$, $SD_{SC\_T1} = 23.5$) than in the ECC group (d = 6.45; r = -0.95; $M_{ECC\_H7} = 56.2$, $SD_{ECC\_H7} = 16.9$; $M_{ECC\_T1} = 201.3$, $SD_{ECC\_T1} = 29.9$) and the ESC group (d = 6.75; r = -0.96; $M_{ESC\_H7} = 50.1$, $SD_{ESC\_H7} = 12.5$; $M_{ESC\_T1} = 218.3$, $SD_{ESC\_T1} = 32.9$). No differences in effect size for contacts with the tunnels were observed between the ECC and ESC groups.

3.7. Grooming

Time spent on grooming in each test phase was measured.

The analysis showed no significant phase by housing setups interaction (Wilks' Lambda; p = 0.738), but a significant main factor effect for the phase (Wilks' Lambda - F(1,27) = 5.336, p = 0.006; $eta^2 = 0.390$).

A visual data analysis revealed that in laboratory rats, the amount of time spent on grooming was low in all the groups under study throughout the experiment; the values fell within the range of 3.4–32.3 seconds.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent on grooming by all the animals tested between individual trials. A significant decrease in grooming activities was only observed in trial T2/T3 (t(28) = 2.293, p = 0.034; Cohen's d = 0.419).

4. Discussion

The analysis of exploratory behaviour revealed that at the beginning of the experiment, rats maintained in the standard environment were more active in the tunnel zones than their enriched housed counterparts. They spent more time exploring the objects and less time in the starting box and the central zone. This may suggest that rats from the control group deprived of the possibility of interacting with the objects in their home environment exhibited a higher propensity for interactions with a new environment (cf. Fernandez-Teruel et al., 1997; Tanas et al., 2015; Makowska and Weary, 2016). It is also possible that the adaptation to the test environment occurred more slowly in the individuals from this group, and that they spend more time familiarising themselves with the surroundings (Matzel and Sauce, 2017). Nevertheless, at the end of the habituation period, activity levels were almost equal in all study groups, which may point to a similar level of habituation to the experimental arena. After the introduction of novelty to one of the zones, all study groups showed markedly higher exploration of the new objects. Controls, however, spent the least time in the altered zone and the least time exploring the new objects. In addition, the enriched housed rats quickly habituated to the changes, and the level of exploration of the new objects fell in subsequent trials, while the control rats maintained their high exploration levels in that zone throughout subsequent test trials. This suggests an impact of environmental experience on the learning process. Habituation is, after all, a form of learning, which means that slow habituation of response to change in a familiar environment indicates slower modification of behaviour. These findings are in accordance with the results of other studies in which was found that environmental enrichment accelerated habituation to novelty (Schrijver et al., 2012; Zimmermann et al., 2001). A high level of exploration after the introduction of novelty in the control group may confirm the above assumption that this group was characterised by a slower pace of adaptation to change.

Specific differences were also observed in the level of exploratory behaviour in rats maintained in the two enriched setups. In rats from the changeable environment, no significant changes were recorded in the amount of time spent exploring the objects in the zones which remained unaltered throughout the experiment. In rats housed in the stable environment, there was a marked decrease in exploration of objects in the unaltered zone after novelty was introduced in the other one. However, this had no impact on the increase in the amount of time

spent by both groups on exploring the new objects, and the effect size of this increase was comparable in both groups as well.

The lack of specific differences between rats housed in enriched stable and changing environments may suggest that the enriched stable conditions, despite their lack of changeability, were complex and stimulating enough to enable the rats to manipulate the level of environmental complexity and diversity on their own. This possibility of environmental manipulation allowed them to regulate the level of incoming environmental stimulation. Moreover, the animals' locomotor activity in the home environment may have been a significant source of environmental variability for the other individuals. In addition, it may be concluded that in laboratory rats, a highly and constantly changing environment did not change the anxiety level as observed at the behavioural level. This is in line with a widely acknowledged finding (Manosevitz and Pryor, 1975), which indicates that cage dimensions alone are the crucial aspects of environmental enrichment. Those results may be compared with the results of a study conducted on mice (Bailoo et al., 2018). A comparative analysis of behaviour in animals kept in conditions characterised by different enrichment levels showed that the highest welfare rate (lowest level of stress and chronic fear) was observed for mice housed in a highly enriched environment in which additionally a change was introduced every week. Nevertheless, it is difficult to draw any unambiguous conclusions about the specific impact of the change on the animals' welfare. In contrast to the other environmental settings used in the study, the environmental changeability was combined with the largest range of enriching elements, as well as a significantly larger cage available space. It is therefore possible that, as mentioned above, the enlarged cage space was the most significant element in that study as well.

The absence of differences in the level of exploration between the animals from the stable and changeable environments may also be linked to the relatively quick extinguishment of behaviours reinforced by intrinsic reinforcement and the quick habituation of curiosity in conditions of constant change (Tarou and Bashaw, 2007). This assumption may be supported by the fact that the animals were housed in an enriched environment for a long time, while the changes to the environment, albeit constant, were of similar nature. It may be concluded, therefore, that in a setting characterised by long-lasting environmental enrichment, the changeability of the environment plays no major role, at least with respect to exploratory behaviours, general activity level and the pace of habituation to the change encountered in that environment.

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References

Abou-Ismail, U.A., Burman, O.H., Nicol, C.J., Mendl, M., 2010. The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. Behav. Process. 85 (2), 172–180.

Bailoo, J.D., Murphy, E., Boada-Saña, M., Varholick, J.A., Hintze, S., Baussière, C., et al., 2018. Effects of cage enrichment on behavior, welfare and outcome variability in female mice. Front. Behav. Neurosci. 12.

Baroncelli, L., Braschi, C., Spolidoro, M., Begenisic, T., Sale, A., Maffei, L., 2010. Nurturing brain plasticity: impact of environmental enrichment. Cell Death Differ. 17 (7), 1092–1103.

Bassett, L., Buchanan-Smith, H.M., 2007. Effects of predictability on the welfare of captive animals. Appl. Anim. Behav. Sci. 102 (3–4), 223–245.

Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., Yaniv, I., Pick, C.G., 2004. Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. Eur. J. Neurosci. 20 (5), 1341–1347.

Benus, R.F., Koolhaas, J.M., Van Oortmerssen, G.A., 1987. Individual differences in behavioural reaction to a changing environment in mice and rats. Behaviour 100 (1), 105–121.

Bloomsmith, M.A., Brent, L.Y., Schapiro, S.J., 1991. Guidelines for developing and managing an environmental enrichment program for nonhuman primates. Lab. Anim. Sci. 41 (4), 372–377.

Camel, J.E., Withers, G.S., Greenough, W.T., 1986. Persistence of visual cortex dendritic alterations induced by postweaning exposure to a "superenriched" environment in rats. Behav. Neurosci. 100 (6), 810–813.

Chorpita, B.F., Barlow, D.H., 1998. The development of anxiety: the role of control in the early environment. Psychol. Bull. 124 (1), 3–21.

Cramer, K.M., Nickels, J.B., Gural, D.M., 1997. Uncertainty of outcomes, prediction of failure, and lack of control as factors explaining perceived helplessness. J. Soc. Behav. Pers. 12 (3), 611–630.

D'Aquila, S.P., Peana, A.T., Carboni, V., Serra, G., 2000. Exploratory behaviour and grooming after repeated restraint and chronic mild stress: effect of desipramine. Eur. J. Pharmacol. 399 (1), 43–47.

Dahlqvist, P., Rönnbäck, A., Bergström, S.A., Söderström, I., Olsson, T., 2004. Environmental enrichment reverses learning impairment in the Morris water maze after focal cerebral ischemia in rats. Eur. J. Neurosci. 19 (8), 2288–2298.

Fernandez-Teruel, A., Escorihuela, R.M., Castellano, B., Gonzalez, B., Tobena, A., 1997. Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: focus on the Roman rat lines. Behav. Genet. 27 (6), 513–526.

Francis, D.D., Diorio, J., Plotsky, P.M., Meaney, M.J., 2002. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J. Neurosci. 22 (18), 7840–7843.

Friard, O., Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol. Evol. 7 (11), 1325–1330.

Frick, K.M., Fernandez, S.M., 2003. Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. Neurobiol. Aging 24 (4), 615–626.

Gardner, E.B., Boitano, J.J., Mancino, N.S., D'Amico, D.P., Gardner, E.L., 1975. Environmental enrichment and deprivation: effects on learning, memory and exploration. Physiol. Behav. 14 (3), 321–327.

Genaro, G., Schmidek, W.R., 2001. Exploratory activity of rats in three different environments. Ethology. 106 (9), 849–859.

Gibson, J.J., 2014. The Ecological Approach to Visual Perception: Classic Edition. Psychology Press, Routledge.

Hebb, D.O., 1946. On the nature of fear. Psychol. Rev. 53 (5), 259.

Jankowsky, J.L., Melnikova, T., Fadale, D.J., Xu, G.M., Slunt, H.H., Gonzales, V., Younkin, L.H., Younkin, S.G., Borchelt, D.R., Savonenko, A.V., 2005. Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. J. Neurosci. 25 (21), 5217–5224.

Job, R.F.S., 2006. The effects of uncontrollable, unpredictable aversive and appetitive events: similar effects warrant similar, but not identical, explanations? Integr. Phys. Beh. Sci. 37 (1), 59–81.

Katz, R.J., Roth, K.A., Carroll, B.J., 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci. Biobehav. R. 5, 247–251.

Kolb, B., Whishaw, I.Q., 1998. Brain plasticity and behavior. Annu. Rev. Psychol. 49 (1), 43–64.

Komorowska, J., Pisula, W., 2003. Does changing levels of stress affect the characteristic of grooming behavior in rats? Int. J. Comp. Psychol. 16 (4), 237–246.

LaDage, L.D., 2015. Environmental change, the stress response, and neurogenesis. Integr. Comp. Biol. 55 (3), 372–383.

Leonard, B., McNaughton, B.L., 1990. Spatial representation in the rat: conceptual, behavioral, and neurophysiological perspectives. In: Kesner, Raymond P., Olton, David S. (Eds.), Neurobiology of Comparative Cognition, pp. 363–422.

Lewis, M.H., 2004. Environmental complexity and central nervous system development and function. Dev. Disabil. Res. Rev. 10 (2), 91–95.

Makowska, I.J., Weary, D.M., 2016. Differences in anticipatory behaviour between rats (*Rattus norvegicus*) housed in standard versus semi-naturalistic laboratory environments. PLoS One 11 (1), e0147595.

Manosevitz, M., Pryor, J.B., 1975. Cage size as a factor in environmental enrichment. J. Comp. Physiol. Psychtr. 89 (6), 648–654.

Matzel, L.D., Sauce, B., 2017. Individual differences: case studies of rodent and primate intelligence. J. Exp. Psychol. Anim. L 43 (4), 325–340.

Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. 44 (2), 229–243.

Pietropaolo, S., Branchi, I., Cirulli, F., Chiarotti, F., Aloe, L., Alleva, E., 2004. Long-term effects of the periadolescent environment on exploratory activity and aggressive behaviour in mice: social versus physical enrichment. Physiol. Behav. 81 (3), 443–453.

Pisula, W., 2003. The Roman high- and low-avoidance rats respond differently to novelty in a familiarized environment. Behav. Process. 63 (2), 63–72.

Pisula, W., 2004. Response to novelty in low-stress conditions in rats. Pol. Psychol. Bull. 35 (2), 99–103.

Pisula, W., Siegel, J., 2005. Exploratory behavior as a function of environmental novelty and complexity in male and female rats. Psychol. Rep. 97, 631–638.

Pisula, W., Ostaszewski, P., Matysiak, J., 1992. Effects of physical environment and social experiences on stimulus seeking behavior and emotionality in rats (*Rattus Norvegicus*). Int. J. Comp. Psychol. 5, 124–137.

Pisula, W., Stryjek, R., Nałecz-Tolak, A., 2006. Response to novelty of various types in laboratory rats. Acta Neurobiol. Exp. 66 (3), 235–243.

Pisula, W., Turlejski, K., Stryjek, R., Nałęcz-Tolak, A., Grabiec, M., Djavadian, R.L., 2012. Response to novelty in the laboratory Wistar rat, wild-captive WWCPS rat, and the gray short-tailed opossum (*Monodelphis domestica*). Behav. Process. 91 (2), 145–151.

Poucet, B., Benhamou, S., 1997. The neuropsychology of spatial cognition in the rat. Crit. Rev. Neurobiol. 11 (2-3).

Rietveld, E., Kiverstein, J., 2014. A rich landscape of affordances. Ecol. Psychol. 26 (4),

325–352.
Rosenzweig, M.R., Bennett, E.L., 1996. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav. Brain Res. 78 (1), 57–65.
Schrijver, N.C., Bahr, N.I., Weiss, I.C., Würbel, H., 2002. Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacol. Biochem. Be. 73 (1), 209–224.
Staddon, J.E., 2016. Adaptive Behavior and Learning. Cambridge University Press.
Tanaś, Ł., Pisula, W., 2011. Response to novel object in Wistar and wild-type (WWCPS) rats. Behav. Process. 86, 279–283.
Tanas, L., Ostaszewski, P., Iwan, A., 2015. Effects of post-weaning social isolation and environmental enrichment on exploratory behavior and anxiety in Wistar rats. Acta Neurobiol. Exp. 75, 72–79.
Tarou, L.R., Bashaw, M.J., 2007. Maximizing the effectiveness of environmental enrichment: suggestions from the experimental analysis of behavior. Appl. Anim. Behav. Sci. 102 (3-4), 189–204.
Thor, D.H., Harrison, R.J., Schneider, S.R., Carr, W.J., 1988. Sex differences in investigatory and grooming behaviors of laboratory rats (*Rattus norvegicus*) following exposure to novelty. J. Comp. Psychol. 102 (2), 188.
van Erp, A.M.M., Kruk, M.R., Meelis, W., Willekens-Bramer, D.C., 1994. Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening. Behav. Brain Res. 65 (1), 47–55.
Wasserman, E.A., Young, M.E., Cook, R.G., 2004. Variability discrimination in humans and animals: implications for adaptive action. Am. Psychol. 59 (9), 879.
Widman, D.R., Rosellini, R.A., 1990. Restricted daily exposure to environmental enrichment increases the diversity of exploration. Physiol. Behav. 47 (1), 57–62.
Zimmermann, A., Stauffacher, M., Langhans, W., Würbel, H., 2001. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav. Brain Res. 121 (1-2), 11–20.