Social housing promotes cognitive function and reduces anxiety and depressive-like behaviours in rats

Jaroslav Nádeníček[1,2], Eva Voslářová[1], Veronika Vojtkovská[1], Zoran Todorović[3], Vladimír Večerek[1]

[1]University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Protection and Welfare and Veterinary Public Health, Brno, Czech Republic
[2]Masaryk University, Faculty of Medicine, Department of Physiology, Brno, Czech Republic
[3]University of Belgrade, Faculty of Medicine, Department of Pharmacology, Clinical Pharmacology and Toxicology, Belgrade, Serbia

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Abstract

The aim of the study was to assess the impact of social isolation of rats in the post-weaning period using behavioural tests aimed at assessing cognitive function, anxiety, and depressive-like behaviours. The monitoring was performed in male Wistar rats which were housed after weaning either individually (n = 8) or in pairs (n = 8) for 33 days. In the open field, rats kept in isolation reared less often ($P < 0.05$) than pair-housed rats. In the elevated plus-maze test, pair-housed rats entered the open arm more frequently ($P = 0.002$) and stayed in the closed arm less often ($P = 0.019$) compared to rats housed in isolation. In the forced swim test, climbing was seen more frequently ($P = 0.016$) in pair-housed rats whereas immobility was more common ($P = 0.006$) in rats housed individually. In the novel object recognition test, the pair-housed rats preferred ($P = 0.014$) the novel object whereas there was no difference ($P = 0.107$) in time spent by exploring familiar and novel objects in rats housed in isolation. Furthermore, juvenile rats housed for 33 days in isolation showed higher ($P = 0.003$) body weight gain during the monitored period than rats housed for the same period in pairs. Our findings are important not only in terms of assessing the impact of rat housing on their mental and physical development but also in terms of the accurate interpretation of the results of other experiments where the rat is used as a model organism.

Isolation, pair housing, laboratory rodents, behavioural tests

Laboratory rats are widely used in research. The rat (*Rattus* spp.) has been the major model species in many biomedical studies (e.g. Erol et al. 2020; Kang et al. 2020; Mohamed et al. 2020; Holovska et al. 2021; Ozdemir et al. 2021; Temiz et al. 2021). Knowledge of the ethological needs of laboratory rats is a basic pillar of the breeding and use of these animals. Wild rats live primarily in large colonies of a size that depends on the availability of food resources; they can consist of more than 150 individuals (Davis 1953). The rat colonies are usually divided into subgroups of pairs or harems with offspring or single males or females (Calhoun 1963). The way of communication between these subgroups is still unknown, although it is clear that the animals must communicate in some way. An example is their ability to avoid contaminated food etc. Social behaviour in rats is manifested mainly in the burrows that they dig together. According to Telle (1966), rats create shared nesting and food storage sites. This means that for rats, social contact is essential. Interactive behaviour in the form of social play, encounters, and the common search for food is already important at an early age (Hole 1991). Play behaviour is crucial, especially for forming the social organization in a group or for the development of the ability to express and understand intraspecific communication signals (Vanderschuren et al. 1997). Early social isolation causes abnormal patterns of social or aggressive

Address for correspondence:
Prof. Ing. Eva Voslářová, Ph.D.
Department of Animal Protection and Welfare and Veterinary Public Health
Faculty of Veterinary Hygiene and Ecology
University of Veterinary Sciences Brno
Palackého tř. 1946/1, Brno 612 42, Czech Republic
E-mail: voslarovae@vfu.cz
http://actavet.vfu.cz/

behaviour (Gerall et al. 1967; Einon et al. 1991). Understanding the negative effects of social isolation at an early age is crucial for understanding the functioning of neural processes. Social isolation may not always be associated with an increase in anxiety-like behaviour (Hall 1998). Consideration should be given to whether the change in anxiety-like status is caused by long-term isolation or by isolation during a critical period of development. Arakawa (2005, 2007) demonstrated that resocialization of isolated rats did not reduce anxiety-like elements in their behaviour. However, if isolation did not occur until late adolescence, it did not lead to the occurrence of anxiety-like elements. According to Arakawa (2005), early isolation evokes elements of anxiety behaviour, thus it is essential to provide suitable social housing for rats, especially at an early age. Socially isolated rats also show symptoms of depression, such as impaired REM sleep (Benca et al. 1996).

The aim of the study was to assess the impact of social isolation of rats in the post-weaning period using behavioral tests aimed at assessing cognitive function, anxiety, and depressive-like behaviours.

Materials and Methods

Animals and their housing

The monitoring was performed in Wistar rats (*Rattus norvegicus*). The rats stayed with their mothers until weaning. All litters were reared in the same room under the same conditions (a difference in the time of birth of individual litters was 1 to 2 days). At the age of 21–23 days, male rats were moved and housed in standard cages (40 × 26 × 20 cm) (l × w × h) in the room accredited for housing of laboratory animals under standardized conditions: light cycle 12/12 h (light/dark), temperature 21 ± 1.20 °C, relative humidity 78–87%. They were provided with standard pellet feed (Altromin Spezialfutter GmbH & Co., D) and water *ad libitum*. For the purposes of the study, rats (n = 16) were randomly divided into two groups. Eight rats were housed individually (individual housing) and eight rats were housed in pairs (social housing). Individually housed rats had no visual contact with other rats, however, they remained in olfactory and auditory contact. Both groups were kept in the same room for 33 days, including the duration of the behavioural tests. During the first week, the rats were left undisturbed to permit for acclimatization to the housing conditions. Besides conducting behavioural tests, all rats were weighed regularly once a week. The animals were handled in compliance with relevant legislation and upon obtaining the consent of the Ethics Committee.

Open field test

The test was developed to measure spontaneous locomotor activity in rodents (Seibenhener and Wooten 2015). The test was performed repeatedly on days 8 and 30 to compare the possible development of rat behaviour depending on the housing. Before the test, rats were moved to the test room and acclimated for 20 min. At the beginning of the test, each animal was placed in a square plastic arena (50 × 50 × 40 cm) (l × w × h), which was divided into a peripheral zone measuring 20 cm from the edge of the arena walls and a central zone (square 30 × 30 cm). The rat was placed in the centre of the arena and its behaviour was recorded employing a video camera and subsequently evaluated. Time spent in the central square, frequency of rearing (behaviour in which the rat stands on its hind legs), and grooming were measured. The test lasted 5 min. Upon its completion, the animal was returned to its home cage and the plastic arena was cleaned with a 70% ethanol solution.

Elevated plus-maze test

The test was performed on day 31. The elevated plus-maze was made of grey PVC material, consisting of two opposite open arms (50 × 10 cm) and two closed arms (50 × 40 × 10 cm) (l × h × w) connected by a central square (10 × 10 cm) (Pellow et al. 1985). The maze was located 50 cm above the floor in the test room with dim lighting. Each animal was acclimatized to the test room for 20 min before testing, then placed in the central square facing a closed arm, and its behaviour was recorded by a video camera for 5 min. Time spent by each rat in the open and closed arms was assessed. After each test, the maze was cleaned with a 70% ethanol solution.

Forced swim test

The forced swim test was performed according to Porsolt et al. (1977) on day 32. The principle of the test was to put the rats in a hopeless and potentially life-threatening situation when they were individually placed in a transparent cylindrical glass tank filled with lukewarm water (23–25 °C). The height of the water column was 30 cm, thus the rat could not touch the bottom of the tank, either with its feet or tail. The test itself was preceded by a pre-test, in which the animal was placed in the tank for 15 min to get acquainted with the environment and to understand that the situation was hopeless. After 24 h, the 5-min forced swim test was conducted. Behaviour of rats during the swim exposure was recorded by means of a video camera and subsequently evaluated, time spent by swimming (horizontal movement on the surface and diving), climbing (vertical attempts to climb the walls), and immobility (floating or using minimal actions to keep the head above water) was measured for each rat.

Novel object recognition test

The test was performed on day 33. First, a pre-test was performed, in which each rat was placed in an arena (50 × 50 × 40 cm) (l × w × h) for 5 min to familiarize itself with the environment (training session). In the arena, two identical objects (glasses) were at a sufficient distance from the walls of the arena and each other so that they did not prevent the rat from moving freely over the entire area. The training session was not recorded. After 5 min, the animal was returned to its home cage for 3 min (retention time). After the retention time, the rat was moved back to the arena and presented with one familiar object (a glass) and one novel object (a can) for 5 min (testing session). Behaviour of each rat during the testing session was recorded by a video camera and subsequently evaluated. Time spent by exploring (approaching and sniffing) the familiar and novel objects was measured for each rat. After each test, the arena and all objects were cleaned with a 70% ethanol solution.

Statistical analysis

Statistical analysis was performed in the statistical program Unistat 6.5 for Excel (Unistat Ltd., UK). The normality of the data was tested by Shapiro-Wilk test. The difference in body weight gain of rats housed individually and in pairs was tested by an unpaired *t*-test. The differences in time spent in the central square and frequency of rearing and grooming in the open field between rats housed individually and in pairs were tested by unpaired *t*-test, and repeatedly on days 8 and 30 by paired *t*-test in the same animals. Paired *t*-test was also used to compare the time spent in the open and closed arms by individually or pair-housed animals in the elevated plus-maze test. Unpaired *t*-test was used to compare time spent in the open (or closed) arm between individually and pair-housed animals. Differences in the duration of swimming and climbing between individually and pair-housed animals in the forced swim test were evaluated by unpaired *t*-test; comparison of the time spent immobile was performed by Mann-Whitney test. Wilcoxon test was used in the novel object recognition test to compare the difference in time spent exploring familiar and novel objects by individually and pair-housed rats. A value of $P < 0.05$ was considered significant.

Results

Body weight gain

Juvenile rats housed for 33 days in isolation showed higher ($P = 0.003$) body weight gain during the monitored period than rats housed for the same period in pairs.

Open field test

Rats kept in isolation showed lower ($P = 0.044$) activity in the central square on day 8 of individual housing compared to day 30, the animals stayed closer to the arena wall. In pair-housed rats, no difference ($P = 0.574$) was found in time spent in the central square between days 8 and 30 of social housing (Fig. 1).

Comparison of the two groups shows that on day 8, pair-housed rats spent more ($P = 0.010$) time in the central square than rats kept in isolation. On day 30, there was no difference ($P = 0.389$) in the time spent in the central square between individually and pair-housed rats.

On day 8, rearing was seen less often ($P = 0.045$) in individually housed rats than in pair-housed rats (Fig. 2). Rearing was more frequent ($P = 0.010$) in pair-housed rats also on day 30. The frequency of rearing did not differ between days 8 and 30 either in individually housed rats ($P = 0.137$) or in pair-housed rats ($P = 0.183$). There was no difference in the time spent with grooming in the arena between individually and pair-housed rats ($P > 0.05$).

Elevated plus-maze test

Rats kept in isolation spent less ($P < 0.001$) time in the open arm than in the closed arm. Likewise, socially housed rats stayed longer ($P = 0.045$) in the closed arm than in the open arm (Fig. 3). However, compared to rats kept in isolation, pair-housed rats entered the open arm more often ($P = 0.002$) and spent less time in the closed arm ($P = 0.019$).

Forced swim test

The results of the forced swim test are shown in Fig. 4. The time spent swimming did not differ ($P = 0.910$) between individually and pair-housed rats. Climbing was more ($P = 0.016$) frequent in pair-housed rats. The time spent immobile was longer ($P = 0.006$) in individually housed rats.

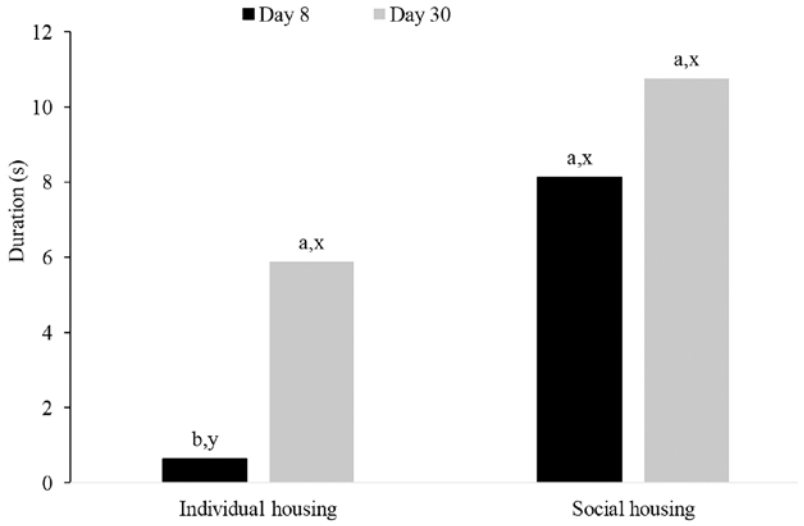

Fig. 1. The difference in time spent in the central square between individually and socially housed rats.

[a,b] – time spent in the central square on different days of testing within the same housing system with no common superscript differ significantly ($P < 0.05$)
[x,y] – time spent in the central square between individually and socially housed rats on the same day of testing with no common superscript differ significantly ($P < 0.05$)

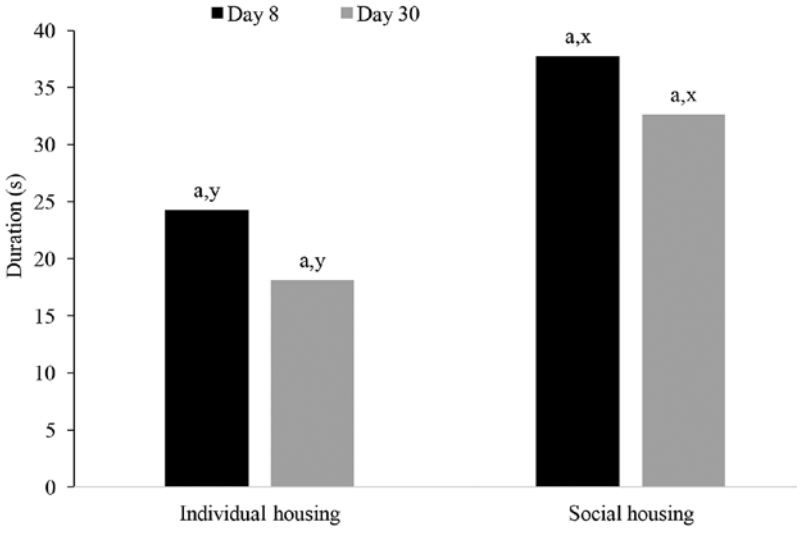

Fig. 2. The difference in frequency with which the rat stood on its hind legs in the field (rearing) between individually and socially housed rats.

[a,b] – time spent by rearing on different days of testing within the same housing system with no common superscript differ significantly ($P < 0.05$)
[x,y] – time spent by rearing between individually and socially housed rats on the same day of testing with no common superscript differ significantly ($P < 0.05$)

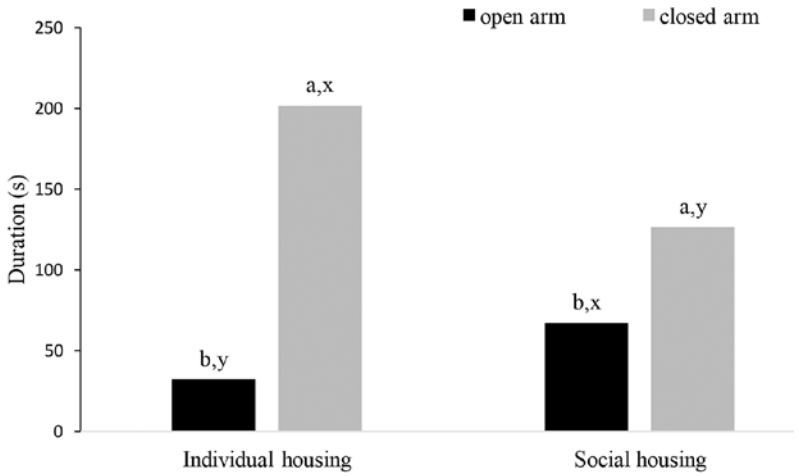

Fig. 3. The difference in time spent in the open arms and the closed arms between individually and socially housed rats.

[a,b] – time spent in the open arms and the closed arms within the same housing system with no common superscript differ significantly ($P < 0.05$)
[x,y] – time spent in the open arms or the closed arms between individually and socially housed rats with no common superscript differ significantly ($P < 0.05$)

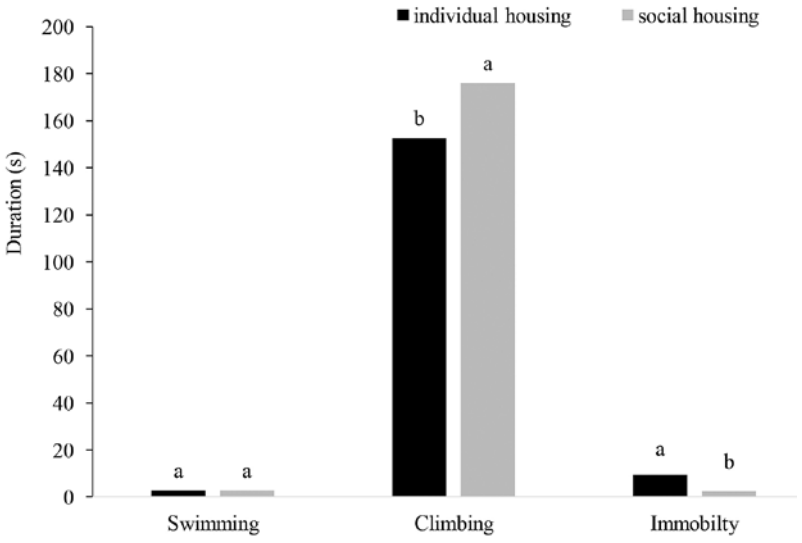

Fig. 4. Duration of selected behavioural elements measured during the forced swim test in individually and socially housed rats

[a,b] Duration of the selected behavioural element with no common superscript differ significantly between individually and socially housed rats ($P < 0.05$)

Novel object recognition test

In rats housed in isolation, there was no difference in the time spent exploring the novel object compared to the familiar object ($P = 0.107$). In contrast, socially housed rats significantly preferred ($P = 0.014$) the novel object over the familiar one (Fig. 5).

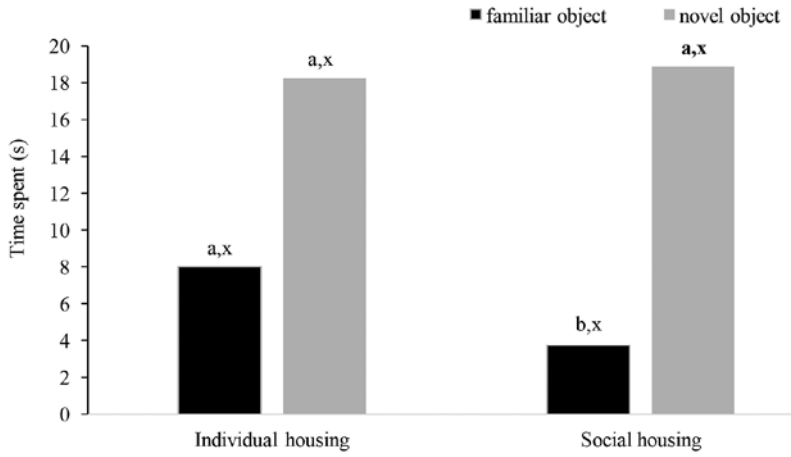

Fig. 5. The difference in time spent exploring the familiar and novel objects between individually and socially housed rats.

[a,b] – time spent exploring the familiar and novel objects within the same housing system with no common superscript differ significantly ($P < 0.05$)
[x,y] – time spent exploring the familiar or novel objects by individually and socially housed rats with no common superscript differ significantly ($P < 0.05$)

Discussion

Estimation of the anxiety level in experimental animals is important in pharmacology and physiology (Sudakov et al. 2013). For this purpose, various anxiety behavioural assays are used in rodents, generally focusing on ethologically relevant behavioural paradigms (Lezak et al. 2017). According to Sudakov et al. (2013), individual anxiety or resistance to emotional stress should be evaluated by several tests since each test might evaluate a different indicator of anxiety level. Therefore, the rats were exposed to several standard tests in our study, namely, the open field test, elevated plus-maze test, forced swim test, and novel object recognition test.

The open-field test was developed to test the emotionality of rodents and evolved as a commonly used tool to assess novel environment exploration and general locomotor activity (Gould et al. 2009). Furthermore, it is used as an initial screen for anxiety-related behaviour in rodents. It is based on the assumption that anxiety involves a conflict between the drive to avoid and the drive to explore an open space. Increased anxiety will result in less locomotion and a preference to stay close to the walls of the field (Ennaceur 2014). In our study, this was evident in rats that were housed individually after weaning and tested on the eighth day of individual housing. Compared to pair-housed rats, they spent significantly less time in the central square. Similarly, Paulus et al. (1998) observed long direct movements along the arena wall, reduced habituation, and predictable movement patterns in the open field in isolated rats. However, in the same test performed in our study on day 30, there was no longer a difference between individually and pair-housed rats in

the time spent in the central square. The results can be explained by habituation to the test arena rather than habituation to individual housing since differences in anxiety levels between individually and socially housed rats were still shown in other indicators and tests performed in our study. Correspondingly, Brenes et al. (2009) documented open-field habituation in rats and found that habituation was impaired in rats housed in isolation whereas environmental enrichment accelerated open-field habituation.

In the open field, we also observed spontaneous rearing behaviour, in which rodents stood on their hind legs to explore the environment. There was a significant difference in the frequency of rearing between individually and pair-housed rats on both test days. The frequency with which the rodent stood on its hind legs in the field was higher in pair-housed rats. Reductions in the number of rearing have been interpreted as heightened anxiety responses (Carli et al. 1989; Lamprea et al. 2008).

Higher anxiety levels in individually housed rats were shown also in the elevated plus-maze test. Compared to rats kept in isolation, pair-housed rats entered the open arm more frequently and spent less time in the closed arm. Greater amounts of time spent in the open arms are interpreted as lower anxiety levels (Lezak et al. 2017). Since previous studies suggest that there are differences in elevated plus-maze behaviour when rodents are exposed to the plus maze on more than one occasion (e.g. Bertoglio and Carobrez 2000; Walf and Frye 2007), the test was performed only once. Rats were exposed to the plus-maze after 30 days of individual or pair-housing to assess the long term effects. It has been documented in previous mice studies that differences in housing conditions may alter behaviour in the elevated plus-maze. Long-term social isolation (unlike short-term, i.e., lasting several minutes, the isolation that experimental animals experience immediately before testing in the laboratory) is considered a stressor and a model of anxiety/depression in mice (Hunt and Hambly 2006; Zhu et al. 2006).

The forced swim test, also known as the behavioural despair test, is used to test for depressive-like behaviour in both mice and rats (Mezadri et al. 2011; Yankelevitch-Yahav et al. 2015). Traditionally, 'floating behaviour' (where the animal remains almost immobile making only the movements necessary to keep its head above water) is used as an indicator to analyse 'hopelessness' and thus depressive-like behaviour. In the test, all animals initially struggle to escape, which manifests behaviourally as vertical attempts to climb the wall. In our study, pair-housed rats exhibited climbing significantly longer compared to individually housed rats. Eventually, the animals adopted a typical posture of immobility (floating in the water) alternated with swimming movements. Immobility was more frequently observed in individually housed rats compared to rats housed in pairs. Rats kept in social isolation exhibited a higher incidence of immobility, suggesting that social isolation leads to disorders similar to depression and anxiety. Socially housed rats were willing to put more effort into getting out of the hopeless situation. Similar findings were made also in experiments performed on mice (Petit-Demouliere et al. 2005). Our study shows that individually housed rats exhibit a depressive-like behaviour potentially affecting their survival in life-threatening situations.

It has been shown that anxiety also disrupts cognitive performance (Maloney et al. 2014), including working memory (Moran 2016). The novel object recognition task is used to evaluate cognition, particularly recognition memory, in rodent models of CNS disorders. This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The choice to explore the novel object reflects the use of learning and recognition memory (Mathiasen and DiCamillo 2010). In our study, an increased interest in the novel object was observed only in rats housed in pairs. In rats housed in isolation, there was no difference in time spent exploring the novel object as compared to the familiar object. The results suggest that social isolation, therefore, reduces cognitive function and impairs cognitive memory.

In addition to the significant impact of the housing system on the results of behavioural tests documenting the occurrence of anxiety and depressive-like behaviours and the impact on cognitive function, differences in body weight gain between individual and pair-housed juvenile rats were also found in our study. Juvenile rats housed in isolation showed higher weight gain during the monitored period than rats housed in pairs for the same period. Our study was conducted in the period of rapid growth. In male Wistar rats, initial rapid growth is observed before 60 days of age, after that, bodyweight gain occurs at a slower rate (Novelli et al. 2007). Our results show that in juvenile rats, the type of housing affects not only mental development but also physical growth. Increased body weight gain in individually housed rats may be due to the fact that access to food is not restricted by other animals housed together (Lopak and Eikelboom 2004). However, excessive food intake may also be a coping mechanism in animals exposed to chronic stress or depression (Koob et al. 1989; Dandekar et al. 2008). An increased weight gain week 4 of housing in social isolation was reported also by Nakhate et al. (2010). Consequently, Lopak and Eikelboom (2000) and O'Connor and Eikelboom (2000) documented that pairing rats after a period of individual housing suppressed feeding. Body weight is the primary outcome in some studies (Wang et al. 2004) or is measured as an indicator of the animals' overall health (Hoffman et al. 2008); deviation from an expected body weight indicates abnormalities and is often used as an indicator of animal distress (Talbot et al. 2020), pain, and discomfort (Morton and Griffiths 1985; Baumans et al. 1994). Thus, the knowledge of factors affecting the body weight of laboratory rats is extremely important. Moreover, the body weight of animals can affect drug metabolism, gene expression, metabolic indicators, and other dependent variables measured in animal studies (Ghasemi et al. 2021).

In conclusion, the type of housing has a significant impact on the mental and physical development of rats. The results of our study show that social isolation in juvenile age affects the results of behavioural tests aimed at assessing cognitive function and the occurrence of anxiety and depressive-like behaviours. In addition, it also leads to a higher body weight gain. Our findings are important also in terms of the accurate interpretation of the results of other experiments where the rat is used as a model organism. The impact of anxiety and depression on physical structures in the brain and thus the central control centre of the nervous system has been well documented and thus may affect the results of a large variety of experiments performed on rats.

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