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SOCIAL BEHAVIOUR ANALYSIS OF WISTAR LABORATORY RATS IN THEIR ENVIRONMENT

ANALIZA COMPORTAMENTULUI SOCIAL AL ȘOBOLANILOR DE LABORATOR WISTAR ÎN MEDIUL LOR DE VIAȚĂ

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ABSTRACT

The aims of this study were to record and identify normal patterns of behaviour in two groups of rats (one having 30 rats and the other one having 40 rats) housed in laboratory cages, analyse the frequency of each behavioural pattern, and compare the results with the current findings in the scientific literature. Based on our ethogram, some of the most frequent behaviours that occurred during a 48-hour timeframe (24 hours in September and 24 hours in December) were: huddling and resting, feeding and drinking, grooming, sniffing and exploring, and finally upright standing and climbing. Results based on our findings suggested that there are no significant differences (P value equals 0.9245) according to the T-test and between the two analysed groups of laboratory rats. In conclusion, these findings suggest that laboratory cages may interfere significantly with rats' natural behavioural characteristics, therefore potentially compromising their overall welfare status.

Keywords: laboratory rats, social behaviour, welfare

REZUMAT

Obiectivele acestui studiu au fost de a înregistra și de a identifica modelele normale de comportament la două grupuri de șobolani (unul având 30 de șobolani și celălalt 40 de șobolani) adăpostiți în cuști de laborator, de a analiza frecvența fiecărui model de comportament și de a compara rezultatele cu descoperirile actuale din literatura științifică. Pe baza etogramei noastre, unele dintre cele mai frecvente comportamente care au avut loc în decurs de 48 de ore (24 de ore în septembrie și 24 de ore în decembrie) au fost: înghesuiala și odihna, hrănirea și băutul, toaletarea, adulmecarea și explorarea și statul în picioare și cățăratul. Rezultatele bazate pe constatările noastre au sugerat că nu există diferențe semnificative (valoarea P este egală cu 0,9245) în conformitate cu testul T și între cele două grupuri de șobolani de laborator analizate. În concluzie, aceste constatări sugerează că cuștile de laborator pot interfera în mod semnificativ cu caracteristicile comportamentale naturale ale șobolanilor, ceea ce ar putea compromite starea lor generală de bunăstare.

Cuvinte cheie: șobolani de laborator, comportament social, bunăstare

The laboratory rat currently used in several experimentation facilities across the globe is represented by a domesticated form of the species *Rattus norvegicus*, also known as brown Norway rat. This genus contains approximately 66 species, among them *Rattus rattus*, or the black rat, being the most known. Although most species of the genus *Rattus* are indigenous to subtropical and tropical regions of the globe, both species can be widely found on all continents (16). The beginnings of rat breeding (*Rattus norvegicus*) for experimentation purposes in Europe started nearly 2 centuries ago, being the first mammal ever domesticated for scientific purposes (23).

In the past, laboratory rats were housed in various types of living conditions and standard laboratory cages designed from all kinds of materials. However, during the years, most countries laid down rules and useful guidelines for the accommodation and care of laboratory animals in general. Such an example can be the European Directive for the Protection of Animals Used for Experimental and Scientific Purposes, which was ultimately updated in 2010, under the European Directive 2010/63/EU (16). Additionally, Regulation (EU)2019/1010 introduced a new level of transparency, aiming to accelerate progress towards later on replacing animal use for scientific purposes. The main objective of the Directive was to introduce the principle of the 3Rs, also known by many scientists and students as: replacement, reduction, and refinement (32). Replacement can be defined as the overall methods, strategies, and approaches which contribute toward mitigating the use of live animals in any experiment (32). Reduction refers to any approach that finally results in less use of animals for scientific purposes (32). Refinement represents the modification of any procedures or practices from the moment when

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the animal is born until its death in order to minimise as much as possible its suffering and improve its overall welfare status, or, in other terms, by replacing any species that are sentient (e.g. vertebrates) with species considered less sentient (e.g., non-vertebrates) (32). These guidelines include the minimum space allowance (flooring) allocated per individual in relation to their body weight, the number of individuals housed in a cage, and the height minimum, which needs to be assured in order to perform stretching behaviour without any issue. However, despite heterogeneity standards occurring in different countries, according to Büttner (1993) and Pullen (1976), a minimum cage height of 18-20 cm must be provided, despite the fact that rats at the age of 2.5 months need a minimum of 22 cm height, and by reaching the age of maturity, this increases up to 30 cm height.

According to Bergstra et al. (2016), the animal husbandry sector is struggling with a bad reputation and a significant negative public attitude. The same authors suggest that similar opinions can be consulted in several public debates with the main topic of animal husbandry, e.g., Barnett et al. (2001) and Boogard et al. (2011). However, according to Makowska and Weary (2016), such housing conditions associated with the inability of animals to extend their limbs, therefore restricting their movements, are unacceptable to the general public.

Social Behaviour

Considering the major importance of play in the development of adult social behaviour and their adaptive capacity to various situations, it is important to provide laboratory rats with sufficient space during their first 3-4 weeks after weaning and provide them with a minimum of 0.2 m^2 for a population of seven animals housed in a cage (30). According to Singleton et. al. (2003), rats live in close contact with humans. Although knowledge of wild rats is almost entirely based on a few early studies, due to the fact that formerly used methods and techniques are not considered ethical or safe enough anymore.

Rats play an important role in our world, being a highly adaptive species. Rattus norvegicus represents one of the main species of rats used for experimental purposes. In their natural habitat, they used to burrow and form galleries underground. Their main dietary preferences are represented by vegetables, fruits, and various cereals. They have a very developed sense of smell and climbing ability, being able to easily reach certain peaks (e.g., trees), depending on their age. Unfortunately, their vision is poor; however, it is complementary to their developed sense of smell. Rats are mainly nocturnal animals, being more active during the night. In the wild, especially, but also in laboratory facilities, rats tend to quickly form a hierarchy, while only the most dominant rat is represented by the strongest rat in the group. This organisational form is similar to the ones seen in other species, e.g., lions, being characterised by an alpha male that controls and protects the group while mating several times with different females in the group. If some rats feel threatened, a fighting behaviour pattern may occur, usually represented by biting or different defensive postures; play fighting is also occasionally seen by the laboratory personnel.

Social interactions play an important role in rats' lives, and they involve direct communication, which is a form of intentional transfer of information intra-specifically (26). Rats have very sharp visual, acoustic, and olfactory senses for communication (28). According to Schweinfurth (2020), visual communication, however, plays only a minor role in their social interactions.

Acoustic communication in rats occurs within and above 20 kHz of the human frequency range, with humans being capable of hearing only a defeat cry, which is mostly specific to conspecifics and heterospecifics (7). However, it was found that rats (especially laboratory rats) mostly communicate through ultrasonic sounds, and there are 3 main ultrasonic sounds described by Brudzynski (2009), as the following: an alarm call (frequency: 22 kHz, length: 300–3,000 m/s), a social call (50 kHz, 20–80 m/s), and an isolation call (40 kHz, 80–140 m/s).

Beside acoustic communication, rats are able to transfer valuable information through odours, and such odours have an important role in a wider range of contexts (17, 22). Domesticated rats perform a variety of behaviours in order to establish dominance in certain specific territories by scent-marking them through urine, faeces. or simply rubbing their anogenital area or flanks over an area (15). It is suggested that wild rats of both sexes display similar behaviour for the same reasons (27).

Despite nearly two centuries of captive breeding, laboratory rats can still perform identical behaviours (e.g., burrowing) comparable to those of their wild ancestors if they're placed in a more naturalistic environment; however, such behaviours can be seen even in larger, environmentally enriched cages (8). Although several studies highlighted many differences between wild and domesticated rats, up to this date there is little evidence supporting that domestication has resulted in a loss of behaviours from the species repertoire, suggesting that in nearly all species studied, differences between wild and domestic stocks are only quantitative (20).

According to Makowska and Weary (2016), when given the opportunity, laboratory rats engage in behaviours such us burrowing, climbing,or stretching; however, their overall intensity and duration are complex in comparison with wild rats (*Rattus norvegicus*). The same authors also suggested that although laboratory rats readily engage in burrowing, climbing, and upright standing, up to this date little information is known about the importance of performing such behaviours to rats. Laboratory rats arguably spent less time performing ambulatory activities and exploring the environment in the detriment of resting, as they advanced in age (18). In these specific situations, any other behaviours found unnecessary and energy-consuming will be traded for the main purpose of resting.

In general terms, when an animal is ageing, it tends to be more inactive due to its inability to perform more energy-consuming motions caused by weakening muscles and less coordination capability; therefore, a reduction in different behavioural patterns is more age-related than motivational. For instance, animals must decide what amount of time they would like to allocate for each behavioural pattern, and if the overall daily time decreases, the amount of energy consumed will eventually be much higher (13).

Regarding the amount of time laboratory rats tended to perform upright standing, two studies suggested that on average, rats spend 5-14 % of their daily activity standing taller than 22 cm height and 3-6% standing taller than 27 cm height (11). However, according to the current directive in place, these heights exceed the minimum required standards by the law (EUR-Lex), meaning that an updated version is more than necessary in order to provide them with the best welfare standards possible.

In general terms, laboratory animals are not able to perform their genetically imprinted normal behaviours compared to their conspecifics; therefore, it is essential to understand what we mean under the term ”normal behaviour and welfare'' when we discuss about laboratory rats in order to improve their overall conditions of life. In a study conducted by Makowska and Weary (2016), they investigated the daily frequency, duration, and distribution of burrowing, climbing, and upright standing in laboratory rats throughout the day. The same authors also suggested that as the rats are ageing, the level of their activity is changing as well, potentially influencing their behaviour.

MATERIALS AND METHODS

The two experiments were set to start between April and December 2023; however, the first actual experiment started in September, while the second experiment started in December. The laboratory rats were constantly recorded for a 10-day period for each experiment, from which we analysed a total of 48 hours (day / night time). For recording purposes, therefore, we used a video camera.

For the first assessment, which took place in September, a total of 30 Wistar laboratory rats of mixed genders were allocated for the purpose of this study. All rats had a weight range between 150 and 220 grammes each and an age range between 10 and 12 weeks each. For the second assessment, which took place in December, a total of 40 Wistar laboratory rats of mixed genders were allocated. All rats had a weight range between 190 and 220 grammes each and an age range between 12 and 13 weeks each.

They were housed in a cage located in one room. In the same room, other cages housing different age groups of rats were present. The rats were not moved to this room nor purchased from an authorised supplier; they were bred in the vivarium of the Sarajevo University-Veterinary Faculty. By maintaining them in the same room as their conspecifics, we reduced the potential stress related to transport and other related operations. Access to the room was made possible through a main wooden door equipped with a metal mosquito net designed to stop potential escapes or the entry of other animals.

The first group of rats was housed under reversed lighting, with a lighting period of 12 hours daylight period and 12 hours dark period. Mean room temperature and humidity were 23 ± 30 C and 50 ± 10% during data collection in September. The second group of rats was housed under the same lighting-periods; however, the room temperature and humidity were 20-230 °C and 65 ± 10%. Mechanical ventilation of the room was provided through a ventilator, and non-mechanical ventilation was provided through a window equipped with a metal mosquito net for the same purpose as the entrance door.

Standard laboratory cages were made of horizontal galvanised wire bars to enable standing upright and climbing, and measured 101 x 46 x 34 cm (L x W x H). The lower part of the cage was lined with non-transparent plastic material so that the bottom of the cage could be filled with a 5 cm height sawdust bedding. Cleaning of the cage took place once a week, so the substrate impregnated with urine and faeces could not cause any unnecessary injuries (skin burns or pododermatitis) during the period of the experiment.

Rats had ad libitum access to food (a complete mixture for laboratory rats) and bottled water, but their diet was supplemented regularly with unsweetened cereals, fruits, vegetables, nuts, seeds, and bread.

These experiments were subjected to Bioethical Committee approval, and all animal welfare standards were in line with the provisions stipulated in Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

Data Collection

The cage was filmed continuously with a Xiaomi Smart Camera C300 360°, resolution 2304 x 1296 2K, 3 megapixels, with recording angle of 180°. The camera was mounted to face the cage. Rats were monitored in real-time through the Mi Home application (Wi-Fi-Wi-Fi IEEE 802.11b/g/n 2.4GHz) for the entire period, and the recordings were stored on a Transcend 64 GB SD card. Finally, the recordings were analysed with the VLC media player programme.

After analysing the data, they were introduced in an excel sheet in order to prepare an ethogram of the most common behavioural patterns that occurred during the 48 hours (24 hours in September and 24 hours in December). The whole data was then analysed comparatively, and the interobserver reliability was established. The behavioural patterns of laboratory rats were analysed every 15 minutes (see Results) at a group level, not individually.

We then consulted the scientific literature in order to find out which behavioural patterns can be considered normal in a confined environment represented by laboratory cages, compared to the normal behaviour of rats occurring in the wild.

Burrowing can often be seen in wild rats, repeatedly performing digging behaviour to make their way into the burrow (or outside of it towards the surface), later pushing the soil out with their forepaws; after they reach the end of the burrow, they quickly inspect the surroundings and turn around to eventually repeat the same behavioural patterns (19).

A frequently observed behavioural pattern in this study was climbing. This behaviour is usually preceded by rearing on the hindlegs, usually seen in adult rats, and may represent a form of investigatory response to the environment, although animals that come into contact with an elevated surface may merely reflect a climbing behaviour rather than a preparatory movement for climbing (1).

In this study, upright standing posture was a commonly seen behavioural pattern due to the rats' average age ranging from 10-13 weeks (considering both groups), therefore a much more active population with higher needs for performing their natural behaviours compared to a geriatric group. Also, climbing behaviour can be considered part of the cognitive learning of rats, attempting to perform such moves to investigate the external environment of the cage (through observation, odours, and communication with other conspecifics from other adjacent cages situated in the same room). Lateral stretching, however, was less encountered in our observations; however, it represents an essential part of laboratory rats' behaviour that cannot be excluded.

Statistical Analysis

For the purpose of statistical analysis, we used the T-test (33) and the Microsoft Excel program. Data was collected in such a document and further analysed by using graphical representations, e.g., column, pie, line, and radar charts.

In order to determine the inter-observer reliability, we analysed the patterns of behaviour in both groups of rats separately and during a 24-hour frame. After each 15 minutes of analysis, a variable number of patterns were allocated based on rats' behavioural characteristics seen during that time. In order to simplify all observed behavioural-patterns, we decided to group them in such a manner, so our technique is highly reproducible for further studies (Fig. 1). After analysing a 24-hour interval, the total sum of behavioural patterns seen each 15 minutes was divided by 24 hours, which resulted in the average time spent by the rats per each behavioural pattern (in percentages). Homogeneity of variances was established after the assessment of behavioural patterns identified by evaluator1 1 (E1) and E2 compared to E3.

RESULTS AND DISCUSSIONS

Based on our ethogram (Fig. 1-4) laboratory rats housed in a confined environment tended to spend a significant amount of their time grooming and feeding during the period of reduced activity. Upright standing and climbing were commonly seen; however, did not seem to pose a significant importance for rats during this phase compared to performing various movements, such as walking from one end of the cage to another or simply doing circular movements around it. Huddling and resting seemed to be the least important aspects of rats' activity. However, by the end of the phase and approaching the next phase, the proportion of rats performing this behaviour increased constantly, finally becoming the most important behavioural pattern of their overall activity. This phase was characterised by alternating behaviours of movement, climbing on the cage, upright standing, investigation, and resting. As the peak activity approached, the diversity of behavioural patterns increased considerably. Considering that this study was based on group analysis, we must mention that the sum of behaviours seen in laboratory rats is the sum of all behaviours identified in the group and added individually.The peak period is characterised by a high frequency and intensity of various behavioural patterns performed by laboratory rats in their daily activities.Based on our findings, the most frequent behavioural patterns during 24 hours in the first group of rats are shown in Fig. 1.

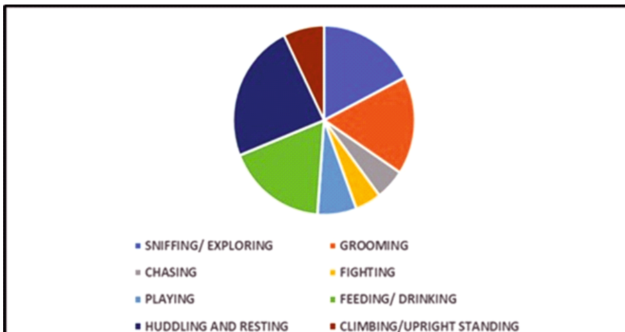

Fig. 1. Behavioural patterns identified in 24 hours - September

During a 24-hour timeframe, the most significant behavioural pattern occurring in laboratory rats was represented by huddling and resting, with a considerable proportion of 72% of their time performing such behaviour. Feeding and drinking seemed to be the second most important aspect in rats' daily activity, with a proportion of 54%, closely followed by sniffing/ exploring (53%), and grooming (51%). A proportion of 22% of their daily activity was spent on climbing/ upright standing, sharply followed by playing behaviour - 21%. Chasing and fighting seemed the least important aspects in laboratory rats' daily activity, amounting to 16% and 14% of their time expenditure, respectively. With regards to the second group of rats, the obtained results over a 24-hour timeframe are shown in Fig. 2.

The most significant behavioural pattern seen was huddling and resting, 82% of the laboratory rats spent their time performing such behaviour. After huddling, grooming seemed to play an important role in rats' daily activity, with a considerable proportion of 66%. Feeding and drinking still represented an important aspect of rats' overall time expenditure - 47%, quite closely followed by sniffing/ exploring - 39%. Climbing/ upright standing, however, was present in 25% of their time, compared to playing - 18%, chasing - 18% and fighting - 17%, the least encountered behavioural patterns. Both groups of rats were further analysed and the results are shown in Fig. 3.

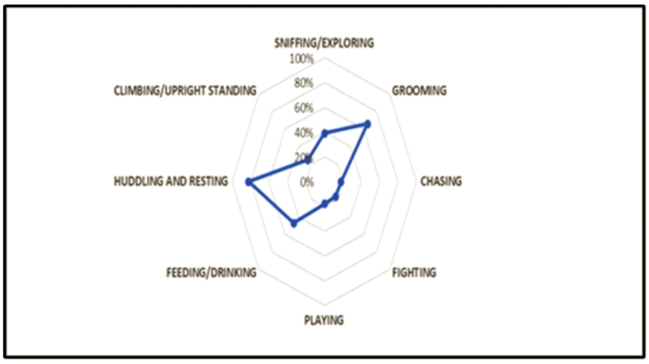

Fig. 2. Behavioural patterns identified in 24 hours - December

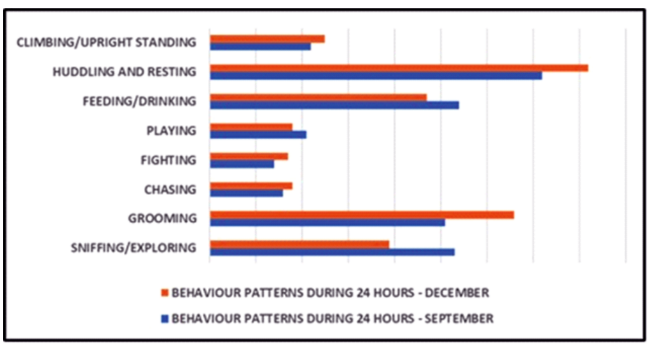

Fig. 3. Behavioural patterns analysis comparatively

From a comparative point of view, huddling/resting seemed to play a less important role in the first group of rats' daily activity - 72% compared to 82% - in the second group. Both groups had different preferences regarding feeding / drinking. While the first group showed a higher interest in feeding/drinking - 54%, the second group expressed a lower interest for that - 47%. Sniffing/exploring behaviour in the first group was performed for a longer period of time - 53%, compared to the second group of rats which only showed such behavioural patterns in 39% of the overall time expenditure. Grooming behaviour was seen 66% of the time over a 24-hour period in the second group of rats, compared to the second group which barely spent half of their time on it - 51%. Differences between both groups of laboratory rats in terms of climbing/upright standing, playing, chasing, and fighting were not statistically significant.

Inter-observer reliability

The validity of our findings was assured by three evaluators; two of them (E1 and E2) analysed the raw data, while the third evaluator (E3) counter-analysed the same data comparatively. A proportion of 84 ± 2% (safety margin) accuracy was achieved, and we considered the final lowest score possible achievable to be 70% in order to avoid compromising the results of this study; however, this was not the case.

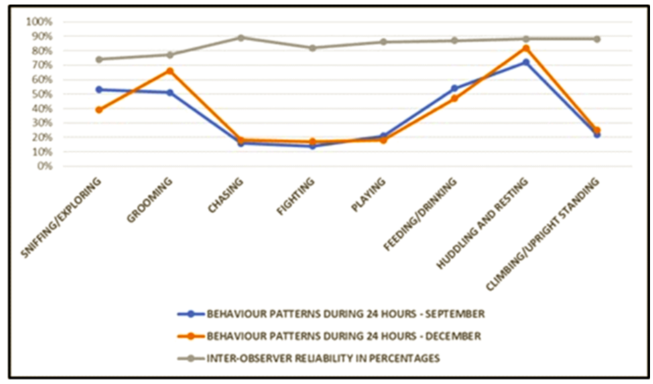

Fig. 4. Inter-observer reliability based on each identified behavioural pattern

Based on our final results of the ethogram, data from both groups of rats were compared using the T-test. This is a test commonly used to check whether the difference between the responses of two target groups is statistically significant or not. Our result showed that the two-tailed P value equals 0.9245. By conventional criteria, this difference is considered to be not statistically significant.

All behaviours were expressed throughout a 24-hour period (once in September and once in December), with much higher activity during the dark phase and peak activity. Most climbing behaviours were considered means of movement, while climbing bouts (head extended vertically and back largely arched) were considered a form of normal behaviour in laboratory rats, which can provide them with important information regarding the external environment as means of an exploratory function. Rats would eventually stand on their lower feet, the jump on cage's bars, and climb up to its ceiling, where they would stay suspended for several seconds up to a minute, sometimes using the bars as so-called ”monkey bars”, by swinging with their forelimbs on the upper bars with their rear limbs hanging downwards (18). Most of these behavioural patterns can therefore be seen frequently in laboratory rats living in a controlled environment. With regards to two ethograms of individual social behaviours encountered in wild rats or rats living under semi-natural conditions (18, 24), we decided to extrapolate some of those behaviours still commonly seen under laboratory conditions.

Sniffing and mobile exploration

Rats are actively involved in exploring the surroundings of the cage, meanwhile gathering valuable information through sniffing the surrounding, e.g., air, bedding, between the cage bars, receiving several external stimuli from their conspecifics and human caretakers present in the room, or just passing by for a short period of time. Their exploration can include sniffing and licking other conspecific's anogenital region as part of their recognition mechanism (2), but also exploration of the entire cage through different patterns of movement, e.g., circling, moving forward or backward, standing on the rear feet meanwhile sniffing, moving certain objects, and burrowing.

Social behaviour (grooming)

More than 50% of laboratory rats' daily activity is spent on grooming and huddling. Grooming was described by us as the behavioural pattern characterised by the cleaning of an individual's own fur through licking its entire body surface. Sometimes nibbling or licking another conspecific's fur with the aid of its forepaws from nose to tail may occur (2). Muzzle washing, chewing, and scratching were common behavioural patterns seen during our investigation.

Chasing

Chasing is a common characteristic of sexual behaviour in rats; however, during this study, chasing was described as the typical behaviour of a rat following one or more conspecifics in order to gather information (external stimuli, e.g., when the caretaker enters the room to feed them), steal food, and engage in play or fight.

Fighting

Rats living in a social group tended to be less aggressive compared to those housed individually. They are very social animal species, constantly supporting each other and sharing information about potential threats, e.g., through vocalisation. However, social hierarchy was highly inherited by laboratory rats, and it's still perpetuated among domestic rat populations. In this study, fighting was not commonly seen; however, few incidents were reported, when a dominant rat had to fight for food by putting its enemy on their back with its thorax and abdomen uncovered by potential threats. Any other fighting behaviours were not recorded during this study.

Playing (sometimes play-fighting)

During a 24-hour period, playing was sporadically seen, being one of the least encountered behavioural patterns among the ones we analysed. Playing is represented by any interaction between two or more individuals that does not cause any harm to each other's health and welfare. In traditional housing systems, the easiest example of rats displaying play behaviour patterns is running on a wheel. It must be mentioned, however, that an individual can attack the nape of its opponent as a form of play fighting (12, 14).

Feeding and drinking

Described by us on a group level as any attempt at food carrying, stealing food from others, food donation, and sharing, including feeding and drinking (e.g., licking the spout of the water tank attached to the cage bars), performed by one or more group members in the same time interval or at different time intervals. While one or more group members performed drinking behaviour, the other might have been eating or the opposite. Rats spend a considerable amount performing such behaviours, especially by youngsters who need high amounts of food in order to compensate for their daily energy requirements. This high energy demand also serves them as an important aspect in the growing and prevention of various diseases by assuring them a healthy lifestyle, of course if the diet is rich in nutrients.

Huddling and resting

Rats lie together with direct body contact, sometimes sleeping one above another (2, 4). Gathering in one place with direct body contact is considered a form of adaptation and survival inherited from their wild ancestors, which can be seen in other animal species as well (e.g., chickens, pigs, wolves, etc.). This behaviour serves them for thermoregulation in the colder periods of the year, provides a higher level of safety to newborns, maintains a high level of attachment between conspecifics, and can also be considered an important aspect of different species' perpetual survival. Resting was considered the lack of any movement performed by laboratory rats in the cage, associated with huddling in one of the corners. Lying in different postures without performing any type of exploration at any time of the day.

Climbing, rearing, and upright standing

Climbing consumes one-fifth of rats' time expenditure; however, the intensity and frequency of such behaviour are very dependent on the animals' age. Young rats tended to climb sporadically during this study, as part of an adaptation to the confined environment represented by laboratory cages. Hoping in other terms is described as individual or repetitive behaviours in order to move forward, explore a larger area (e.g., in the wild), and escape from potential dangers (e.g., in the laboratory facility if running away from humans), while jumping is a similar term but also involves jumping upwards, which can be or not be repetitive, but is usually associated with climbing, e.g., jumping on the cage bars and climbing up to the ceiling. Sometimes more active rats can be suspended with all paws in contact with a vertical surface or the cage ceiling (18). Upright standing is nothing else than just rats standing on their hind legs extended, while forepaws are in a vertical position unsupported by anything or supported by a vertical surface and their back is highly extended (18).

CONCLUSIONS

Laboratory rats housed in a confined environment are subjected to various behavioural and welfare issues; therefore, we humans are solely responsible for their mental and physical well-being.

This study suggested that laboratory rats perform several types of behavioural patterns that can still be seen in the wild; however, their frequency and intensity are possibly lower compared to their conspecifics living in nature. The housing system greatly interferes with rats' natural behaviour, potentially leading to unnecessary stress and diseases in the population.

A proper management system, together with a much larger space allowance and enrichment would be beneficial for rats to perform the natural behaviours they inherited from their ancestors. Further studies to assess laboratory cages' influence on rats' behaviour and welfare are required for a better understanding of what this species needs.

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