Behavior of Male Mice (*Mus musculus*) in Laboratory

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ABSTRACT

The importance of observing the behavior of mice in the laboratory is additional information to compare with their behavior in nature and an effort to increase the welfare of mice (animal welfare) through alleviating the pain and suffering associated with scientific procedures. This study aims to study the behavior of male mice in the laboratory. The method used was ad libitum and focal animal sampling. Based on the ad libitum sampling method, the daily activities of male mice in the laboratory were divided into 9 activities, namely locomotion, grooming, eating, foraging, social, exploration, resting, making nests, and drinking. Based on the focal animal sampling method, the main activity carried out by male mice in the laboratory was locomotion.

Introduction

Behavior is an expression to adapt to different internal and external conditions. Behavior can be described as an animal response to stimuli (Blackshaw et al., 2003). Observation of animal behavior can be done in the field and in the laboratory. Observation of animal behavior in the field is to see its behavior which appears naturally in nature while observing the behavior of animals in the laboratory is to see the behavior that appears in the cage made. Observation of these behaviors serves to compare the behavior of animals that appears in different conditions. The techniques and time rules for observing animal behavior include ad libitum sampling, focal animal sampling, scan sampling, behavior sampling, continuous recording, time sampling. These techniques and time rules are not only used in the field (Blackshaw et al., 2003; Olsson & Sherwin, 2006; Schradin & Pillay, 2003) but also can be used to observe animals in the laboratory, such as mice (Altmann, 1974; Martin & Bateson, 1987).

Mice are the most common vertebrate used in laboratory research, including behavior (Balcombe, 2006). They are more active when placed together. Many studies on the behavior of mice. Most studies of the behavior of mice in the laboratory are based on observing standard behavior under very artificial conditions and taking into account the specific characteristics of animal species, such as their environmental preferences (Olsson, Nevison, Patterson-kane, & Sherwin, 2003). The behavior of mice in the

laboratory, namely feeding (feeding), grooming, foraging (foraging), locomotion (moving), resting (resting), social (social), drinking (drinking), exploration (exploration), and building nests (nest building) (Schellinck, Cyr, & Brown, 2010). The importance of observing the behavior of mice in the laboratory is as additional information to compare with their behavior in nature and as an effort to increase the welfare of mice (animal welfare) through alleviating the pain and suffering associated with scientific procedures. Therefore, this study aims to study the daily activity of male mice in the laboratory using behavioral observation techniques.

Materials and Methods

The tool used in observing the daily activities of male mice (*Mus musculus*) is a watch that has a stopwatch, a learning lamp with a red light bulb (Philips, 15 watts) with a wavelength of 6.60 ± 0.30 nm (Sugito, Sb, Firdausi, & Mahmudah, 2005), and handycam (Sony DCR-SX44E, NP-FV30 number model battery, 7.2 V, 3.6 Wh, 500 mAh, 60x zoom lens) with 4 GB of memory, 75 cm tripod height. Cutting the film using the Windows Movie Maker 2.6 program which can be downloaded for free at http://www.microsoft.com /en-us/download/ details.aspx?id=34.

The sample observed was a group of male mice (*Mus musculus*) strain Deutsche Denken Yoken (DDY) consisting of 3 age ratios, namely 3 months old adults, 1.5 months old adolescents, and 21 days old children. Mice were obtained from the Non-Ruminant and Hope Animal Laboratory, Faculty of Animal Husbandry, Bogor Agricultural Institute.

Observations were made in the cage of the Animal Function and Behavior, Bogor Agricultural Institute, with a total of 6 observations. The size of the cage used is 50 cm x 50 cm x 50 cm as much as 1 fruit with the distance between the researchers and the cage is 1-1.5 m.

The initial study was conducted twice to habituate and find out what activities were carried out by male mice (adults, teenagers, and children) for one night (07.00 pm to 12 pm). Habituation aims that the object observed is accustomed to the presence of observers, and vice versa. Habituation is done by standing near the cage in the same position. During habituation try not to use perfume. Observation of daily activities was carried out for 2 weeks, from 07.00 to 12.00 pm by using a red light. The method used, namely ad libitum and focal animal sampling. The ad libitum sampling method was used at the beginning of the observation to find out what activities were carried out by male mice for one night, by recording all their visible activities. The result is a description of the daily activities of male mice in one night which can be seen in Table 3. In the next observation, the method used is focal animal sampling.

Focal animal sampling, which is observing and recording the activities of one individual mouse at the beginning of a time transition for a certain period of time with individual records always visible. With this method, observers can focus on the activities of each individual. The observation period depends on the nature of the animal and the ability of the observer. Observation of male mice in the laboratory is done one night per individual. The results of this method are the duration and frequency of each activity.

Data analysis was presented descriptively by displaying data in the form of tables, figures, and pie charts regarding the daily activities of male mice in the laboratory as a whole as well as individually.

Results and Discussion

The results of observations of male mice in the laboratory with three male age ratios (adults, juveniles, and infants) with the ad libitum method have 9 activities. These activities can be seen in Table 1.

Table 1. Daily activities of male mice in the laboratory

Activities	Figure	Explanation
Locomotion		Mice walked in the cage lid
Grooming		Mice were grooming its body
Resting		Mice did not carry out activities
Eating		Mice taken and hold food, put food in the mouth, then chewed
Social (Sexual)		Male mice ride female mice

Social (Allogrooming)		Male mice were grooming other male mice
Exploration		Male mice stand
Drinking		Mice attached its mouth to water sources and ingested water
Nesting		Male mice pushed litter with front legs

The results obtained from the ad libitum method, the daily activity of male mice (*Mus musculus*) in the laboratory consisted of grooming, eating, resting, social (sexual and allogrooming), foraging, locomotion, exploration, nesting, and drinking. The focal animal sampling method gives the results of frequency which is the percentage of each daily activity of male mice in the laboratory. Based on this method, the main activity carried out by mice in the laboratory is locomotion (Figure 1).

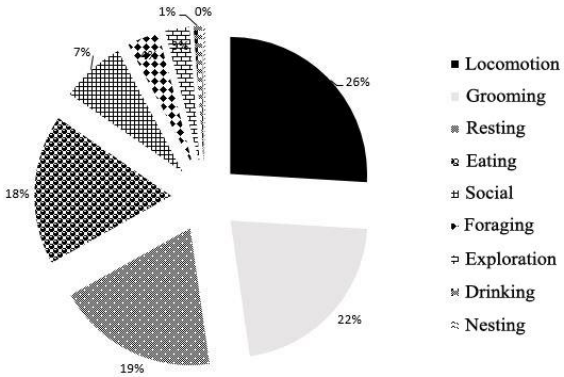

Figure 1. Percentage of behavior of male mice in the laboratory

Locomotion. The largest daily activity of adult and juvenile mice was locomotion, those were 28% and 31%, whereas infant mice were 18% (Figure 2). Locomotion is the activity of moving an individual by walking in a cage and climbing a cage (Koteja, Garland, Sax, Swallow, & Carter, 1999). This shows that male mice are active species. In a period of 4-6 hours, male mice in the laboratory spend more time on locomotion (Van de Weerd et al., 2001).

The percentage of locomotion of juvenile was greater than adult and infant indicated that juvenile was more active. This is evident at night, juvenile rodentia is more trapped than adult (Gurnell, 1982). The locomotion level is influenced by the size of the cage and the number of individuals in the cage (Arakawa, 2005). The size of the cage of 50 cm x 50 cm x 50 cm made juveniles more active in exploring the area than adult.

Besides that, the largest percentage of the infant's daily activity was resting, namely 33% (Figure 2). In resting, mice do not carry out any activities. Infant spends approximately 50% of their time in resting by way of adjacent (Van Loo et al., 2004). This activity can manage their body temperature and makes it safer.

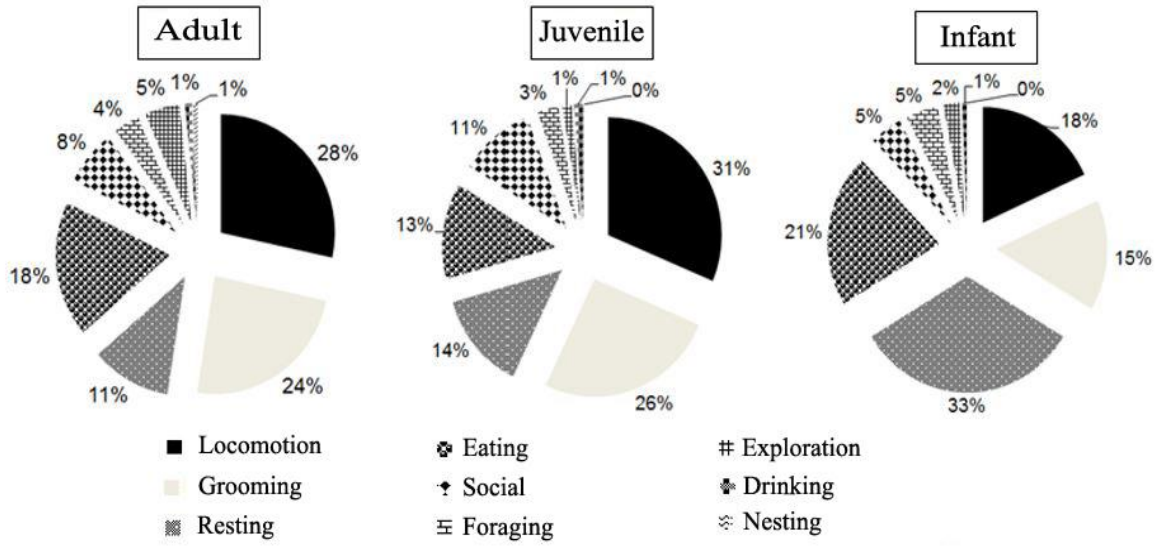

Figure 2. The behavior of each age ratio of male mice in the laboratory.

Grooming

The percentage of grooming activity in each age ratio of male mice in the laboratory was different (Figure 2). The frequency of grooming activity of male mice in the laboratory was more than 15% (Kalueff & Tuohimaa, 2004). Grooming is licking behavior starting from the claws on the forelegs, head, body, legs, genitals, and tail (Denmark et al., 2010). Grooming has 4 phases, namely phase 1 phase 2, phase 3, and phase 4 (Figure 3) (Berridge et al., 2005). The percentage difference in the age ratio of male mice is related to locomotion activities. Grooming occurs spontaneously as a transitional activity between rest and locomotion (Smolinsky et al., 2009). The high level of locomotion of adult and juvenile was suspected to take effect during the transition time so that the grooming activity occurred. Grooming in mice aims to cleanse the body to maintain health by removing detritus that carry parasitic diseases, attracts couples, and avoids predators through the elimination of odors (Feusner et al., 2009).

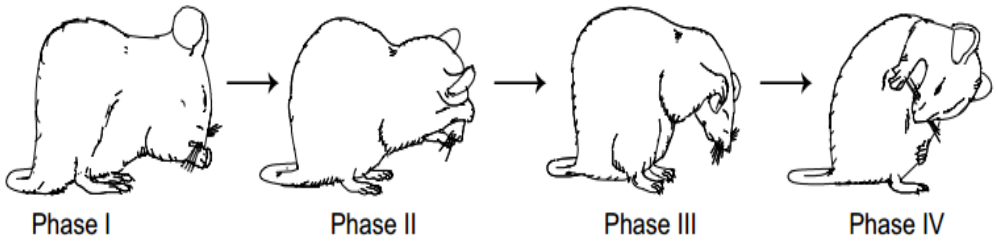

Figure 3. Grooming phase in mice (Berridge et al., 2005)

Resting

Rest is a condition that shows mice do not carry out any activities. Resting activities depend on other activities that have been done by mice (Balcombe, 2006). Based on observations, male mice in the laboratory actively move. The resting frequency of adult and juvenile is directly proportional to locomotion frequency (Figure 2). The higher the frequency of locomotion, the higher the frequency of resting. This is related to the loss of energy that makes mice feel tired so they rest.

Eating

In this activity, the infant had the highest frequency. Mice need energy from food for the maintenance of the body. This activity aims to overcome while no food. Mice must invest more food (stored in the fat form) as a physiological strategy for allocating energy and nutritional intake for growth (Speakman, 2008). In this study, the food given is in the form of pellets.

Social

The social activities seen during the observation of male mice in the laboratory were sexual, genital examination, allogrooming, agonistic, and crowding. Sexual activity is the interaction of male-female aiming for reproduction (Asaba, Hattori, Mogi, & Kikusui, 2014). Female mice attract to male mice because of the smell of male mice that indicates their dominance. Domination is related to the nature of male mice, such as aggressiveness and territoriality (Bronson, 1979). The dominant social status of male mice is assessed by changes in the smell of testosterone found in urine. The dominant urine of male mice is more effective in accelerating the reproductive function of female mice compared to non-dominant mice (Lombardi & Vandenbergh, 1976). Before sexual activity occurs, male mice do a genital examination of the female. A genital examination is more often done by male mice that function to detect the reproductive conditions of the female (Nunn, 2003).

The dominant male mice can be seen through its agonistic activity. Agonistic is aggressive male mice, such as attacking others, chasing, pinching, biting, or aggressive grooming (Figure 4) (Allen, Cragg, Wood, & Pfaff, 2011). Agonistic activity shows the degree of dominance among male mice in the cage. In addition to these activities, allogrooming activity was seen at the time of observation, adult male mice allogrooming into female mice or other male mice. Allogrooming is grooming between individuals of the same sex or different. Allogrooming provides a mechanism for mice to test conformity, such as male mice testing the suitability of female mice to mate (Stopka & Graciasova, 1999).

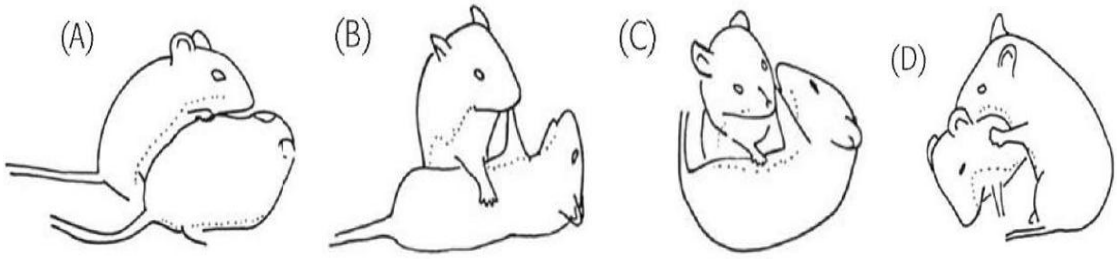

Figure 4. Agonistic (Allen et al., 2011)

Foraging

Foraging activities are activities of mice sniffing the substrate, flipping through litter, etc. (Schellinck et al., 2010). Based on Figure 2, the percentage frequency of each male mice ratio for this activity is not much different. It caused by food spread in the same enclosure area, then the mice will look for it themselves, just as if the mice are in nature but differ in their food variations. The food given to mice in this laboratory is pellets. Foraging activities aim to meet the food needs of mice when there is a food shortage.

Exploration

Exploration activities of male mice in the laboratory are different. The difference in frequency depends on the stage of development of the age of male mice (Arakawa 2005). Adult male mice had the highest exploration activity. This is intended to monitor the territorial area of male mice which later leads to the dominance of the male mice themselves.

Drinking

After eating activities, male mice drink. Similar to eating, drinking also has a function, namely reducing the thirst for mice because the eaten pellets absorb most of the water (Bachmanov, Reed, Beauchamp, & Michael, 2006). Based on Figure 3, the frequency of drinking activity for male mice is the same, which is 1%. In addition to the maintenance of the body in the process of growth and physiology, these two activities are needed to carry out other activities, such as social, exploration, and nest making.

Nesting

Male mice in the laboratory also show nesting activities. This activity is carried out by mice by pushing or collecting material inside the cage to form a collection of material. Tools had an effect on the activity of nesting. In this practical process, the observer gave the material in the form of tissue paper and wood shavings, then put it into the mice cage and saw adult male mice and juvenile male mice were making nests. It has been reported that the use of tissue paper increases the activity of making nests. Nesting materials may have several functions. Mice can regulate their temperature and avoid too much light or hide their partners by building nests. Nests can also be used as a place to live for mice taking refuge and resting (Van de Weerd et al. 1998).

The condition of the observer who is sometimes less fit in making observations might influence the data collection process. This is due to the nature of nocturnal mice while diurnal observers. To overcome this, observers use a 30-minute pause to rest so that they focus on the data retrieval process. In the process of observation, a camcorder that has infrared is needed to record the activity of mice. Observers do not have camcorders that have infrared wavelengths of 8 - 14 µm (Marinelli et al., 2000). Therefore, observers use red light with a wavelength of 6.60 ± 0.30 nm. Based on literature studies, red light can be used as an alternative for observing mice at night.

mice have poor vision and are unable to detect color. Red light is often used to observe mice during nighttime activities (Bedrosian, Vaughn, Weil, & Nelson, 2013).

Conclusion

Based on the ad libitum sampling method, the behavior of male mice in the laboratory is divided into 9 activities, namely locomotion, grooming, eating, foraging, social, exploration, resting, nesting, and drinking. Based on the focal animal sampling method, the main activity carried out by male mice in the laboratory is locomotion.

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