

RELATIONSHIP BETWEEN RABBIT HOUSING SYSTEM AND SEX AND THEIR EXPLORATORY BEHAVIOUR

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Abstract

The aim of the present study was to determine the influence of the housing system and sex of rabbits on their exploratory behaviour. The research material consisted of 66 Termond White rabbits (37 females and 29 males). Until the 35th day of life, the rabbits were kept with their mothers in wooden cages. After weaning, on day 35 the animals were marked with an ear tattoo and randomly assigned to three groups. A group of 14 females and 8 males was placed in three cages located on pasture. The cages, measuring 100 cm × 120 cm × 50 cm, were made of metal mesh with different mesh sizes. In addition, a compartment with enclosed walls and a mesh floor was provided in the cages. Another group of 22 rabbits was housed in pens with deep litter. This group consisted of 9 females and 13 males. Group III (8 males and 14 females) was housed in battery cages used for commercial rabbit rearing, measuring 40 cm × 60 cm × 35 cm. Rabbits from all groups were fed a complete commercial pelleted diet. In addition, group I rabbits kept in the pasture system had unrestricted access to green forage. The open field test was used to investigate exploratory behaviour. The number of fields crossed and the number of rearing events were similar in females and males. Animals housed in deep-litter pens performed significantly more rearing events than animals kept in battery and pasture systems. The rabbit housing system had no significant effect on the number of fields crossed. Differences in the number of rearing events support the hypothesis that the housing system affects animal behaviour.

Keywords: behaviour, exploration, rabbit, open field test

Introduction

The desire to understand animal behaviour has accompanied humans for millennia. Initially, learning the rules governing animal functioning helped people survive in a hostile environment. After centuries and the domestication of many animal species, the roles were reversed: today, by understanding animal behaviour and interactions with the environment, humans can protect animal life.

Every animal placed in a new environment needs to obtain information about it and displays certain emotional responses. The diversity of behaviours observed in such a situation depends on many factors. The most important is the animal's taxonomic affiliation. Individual classes, species and even breeds have characteristic anatomical features. Behaviour is closely related to the structure and function of the nervous system. Certain types of behaviour may also depend on sex. Differences in hormonal regulation between females and males affect their responses (Pisula, 2003). Sense organs enable information from the surroundings to be registered. Different reactions may be observed in the same individual because responses to environmental stimuli change with age; young animals interpret events around them differently. Up to a certain stage of life, these organs are not fully developed, making stressful situations more difficult to cope with. Ageing, in turn, is associated with impaired sensory perception and prolonged reaction time.

In addition to genetic factors, animal behaviour is strongly associated with adaptation to particular living conditions (Pisula, 2003). The housing system has a considerable influence on behaviour because it often imposes a specific pattern of activity and locomotion and teaches appropriate responses to environmental stimuli. The system in which a herd or individual animals are kept is entirely dependent on humans.

The history of systematic research on animal exploration began in the 1950s and 1960s (Whimbley and Denenber, 1967; Bronstein, 1972; Blanchard et al., 1986). Animals of different species show different exploratory behaviours, as demonstrated in studies on opossums (Poran et al., 1993; Wielopolska et al., 2001; Węsierska, 2002), mice (Can et al., 2012a, 2012b; Wietrzyk et al., 2005), rats (Bronstein, 1972; Ostaszewski and Pisula, 1994; Węsierska and Turlejski, 2000; Węsierska, 2002), rabbits (Kowalska and Gugolek, 2007, 2015; Pałka et al., 2015), and domestic cattle (Cramer and Stanton, 2015; Budzyńska et al., 2018). Age, sex and physiological status, including disease, may affect exploratory behaviour (Gray, 1971; Bronstein, 1972; Ostaszewski and Pisula, 1994; Węsierska, 2002; Cramer and Stanton, 2015).

Exploratory behaviour changes with age even within the same species or breed. Very young animals usually do not explore actively because they are accustomed to remaining in the same environment under maternal care. Exposure to a stressful stimulus in behavioural tests often causes immobility. With age, animals become more independent and their responses to environmental factors change and become more learned. More odours cease to be unfamiliar (Pisula, 2003). Opossums remember odours, while novel odours stimulate greater exploration (Poran et al., 1993). The high dynamics and frequency of movements in young animals are associated with play and learning about the environment. Another pattern of exploration is based on sniffing, a behaviour directly related to obtaining information about the surroundings or other individuals (Calhoun, 1963).

Many open field tests have shown no significant differences between the exploratory behaviour of the two sexes. Gray (1971) reported lower anxiety and higher activity in female rodents than in males, but this theory was not confirmed in other studies. Ostaszewski and Pisula (1994) studied rats of both sexes from four strains. In three strains, females showed greater exploratory activity than males, whereas in the fourth strain males were more active and spent more time grooming. Kowalska and Gugolek (2007) found that rabbits initially moved along the peripheral fields in the open field test and only after repeated testing entered the central fields more frequently. No defecation was recorded, but urine marking occurred and was more frequent in males. Similar results were reported by Kowalska and Gugolek (2015): rabbits initially explored the outer fields and entered the inner fields after habituation; 75% of males marked the area with urine and faeces. In Pałka et al. (2015), the number of grooming acts differed only slightly by sex. In opossums, females spent more time exploring the central part of the arena on later days (Węsierska, 2002). In a novel environment, behavioural patterns did not differ substantially by sex. As animals adapt to the surroundings, sex-related factors become more important and exploration becomes more individualised.

Because relatively few open field studies have addressed the relationship between rabbit housing systems and behaviour, the aim of the present study was to determine the effect of housing system and sex on exploratory behaviour in rabbits.

Material and methods

The study involved 66 Termond White rabbits, including 37 females and 29 males. Until 35 days of age, the rabbits were kept in wooden cages in the experimental hall of the Department of Genetics, Animal Breeding and Ethology of the University of Agriculture at Przegorzały. The hall was equipped with lighting and water installations and forced ventilation. After weaning, on day 35, the animals were marked with ear tattoos and randomly assigned to three groups. The first group (14 females and 8 males) was placed in three cages outside the hall (pasture system). The cages were made of metal mesh of different mesh sizes and measured 100 cm × 120 cm × 50 cm. Each cage also contained a compartment with enclosed walls and a metal mesh floor. The next group, comprising 22 animals (14 females and 8 males), was kept in deep-litter pens located in the same hall in which the rabbits had been housed with their mothers. The third group, comprising 13 males and 9 females ($n = 22$), was also housed in the same hall, in battery cages used for commercial rabbit rearing. Each cage measured 40 cm × 60 cm × 35 cm. The animals were fed a complete commercial diet containing 16.5% crude protein, 14% digestible fibre and 10.2 MJ metabolizable energy. Rabbits in the pasture system had unrestricted access to green forage through the mesh floor of the cages.

The open field test was used to examine animal behaviour. A box measuring 2 m × 2 m and 1 m high, painted white on the inside, was constructed. Its floor was divided into 36 equal, consecutively numbered squares. A round blue object was placed in the centre of the field to arouse the animals' curiosity. Each rabbit was observed for 3 minutes. Time was measured with a stopwatch and started when the rabbit was placed on the starting square marked "1". The behaviour of each individual was described using the following indicators:

- number of crossings of designated squares,
- number of rearing events.

A square crossing was counted when all four limbs of the animal were simultaneously within one square. A rearing event was counted when both forelimbs were simultaneously raised above the floor. Statistical analysis was performed using SAS (SAS, 2014). During the open field test, no grooming, urination, defecation or touching of the object in the centre of the field was observed; therefore, statistical analysis was performed only for the number of squares crossed and the number of rearing events. Basic statistics (median, minimum, maximum and

number of observations) were estimated. Normality was then tested and significant deviation from a normal distribution was found for both traits. Consequently, the significance of the effects of sex and housing system was analysed using non-parametric one-way analysis of variance (NPAR1WAY procedure), with the Wilcoxon-Mann-Whitney test for sex, and the Kruskal-Wallis test plus the Dwass, Steel and Critchlow-Fligner (DSCF) method for multiple comparisons among housing systems when statistically justified.

Results

Table 1 presents basic statistics and the test statistic with its significance for the number of fields crossed and rearing events in females and males of group I. The number of fields crossed by males and females did not differ significantly ($P \geq 0.05$), although females were slightly more active than males in this housing system (median 13.5 vs. 10.5). Statistical analyses also showed no significant differences ($P \geq 0.05$) in the number of rearing events, which was similar in males and females.

Table 1. Comparison of the number of fields crossed and rearing events performed by females and males of group I

Behaviour	Sex	n	Median	Minimum	Maximum	S*	P-value**
Number of fields	Female	14	13.5	2.0	21.0	82.5	0.5349
	Male	8	10.5	5.0	16.0		
Number of rearing events	Female	14	2.0	0.0	7.0	80.0	0.4276
	Male	8	1.5	0.0	3.0		

S* – Wilcoxon test statistic; **P-value – probability (significance)

Table 2 presents the median, minimum and maximum, together with the test statistic and its significance, for the number of fields crossed and rearing events by males and females of group II. Females showed a greater tendency to explore than males, although this was not statistically confirmed ($P \geq 0.05$). The analyses indicated no significant sex differences ($P \geq 0.05$) in the number of rearing events, although females performed them more frequently.

Table 2. Comparison of the number of fields crossed and rearing events performed by females and males of group II

Behaviour	Sex	n	Median	Minimum	Maximum	S*	P-value**
Number of fields	Female	9	12.0	4.0	15.0	112.0	0.5873
	Male	13	9.0	4.0	16.0		
Number of rearing events	Female	9	5.0	3.0	14.0	120.0	0.2800
	Male	13	4.0	2.0	12.0		

S* – Wilcoxon test statistic; **P-value – probability (significance)

Table 3 shows the median, minimum, maximum and test statistic with significance for the number of fields crossed and rearing events by males and females of group III. Statistical analysis showed no significant sex differences ($P > 0.05$) in the number of fields explored during the open field test in rabbits housed in the battery system. The medians (12 vs. 7.5) indicate greater locomotor activity in females. No significant differences ($P \geq 0.05$) were found in the number of rearing events, although females again performed slightly more.

Table 3. Comparison of the number of fields crossed and rearing events performed by females and males of group III

Behaviour	Sex	n	Median	Minimum	Maximum	S*	P-value**
Number of fields	Female	14	12.0	1.0	19.0	77.0	0.3207
	Male	8	7.5	3.0	17.0		
Number of rearing events	Female	14	2.0	0.0	6.0	80.5	0.4382
	Male	8	1.0	1.0	4.0		

S* – Wilcoxon test statistic; **P-value – probability (significance)

Table 4 presents basic statistics, test statistic values and significance for comparisons among housing systems in rabbit locomotor activity measured by the number of fields crossed and rearing events. No significant differences were found in the number of fields crossed among the analysed systems ($P \geq 0.05$). However, group I animals more often crossed peripheral/outer squares. The number of rearing events differed highly significantly depending on the housing system ($P < 0.01$).

Table 4. Comparison of the number of fields crossed and rearing events performed by rabbits

Behaviour	Group	n	Median	Minimum	Maximum	T*	P-value**
Number of fields	I	22	12.5	2.0	21.0		
	II	22	9.5	4.0	16.0	1.699	0.428
	III	22	10.5	1.0	21.0		
Number of rearing events	I	22	2.0	0.0	7.0		
	II	22	5.0	2.0	14.0	20.429	<0.001
	III	22	1.5	0.0	6.0		

T* – Kruskal-Wallis test statistic; **P-value – probability (significance)

Table 5 presents comparisons among housing systems for the number of rearing events. Statistical analysis showed that group II differed highly significantly from groups I and III (DSCF test, $P < 0.01$).

Table 5. Dwass, Steel and Critchlow-Fligner (DSCF) test values and significance for comparisons among rabbit housing systems for the number of rearing events

Comparison	DSCF test value	Pr. > DSCF
Group III – Group II	5.7986	0.0001
Group III – Group I	0.4073	0.9553
Group II – Group I	5.1841	0.0007

Pr. > DSCF – probability (significance)

Discussion

The open field test enabled observation of Termond White rabbits of both sexes kept in different housing systems. Statistical analysis showed no significant sex-related differences in exploratory behaviour. The housing system also had no significant effect on the number of fields crossed. The only difference was the peripheral movement of animals kept in the pasture system, probably due to the need for continuous vigilance and observation of the surroundings. The tests did, however, indicate a highly significant effect of housing system on the number of rearing events. Rabbits housed in deep-litter pens explored most intensively by rearing. This pattern may have become established because the pen walls prevented observation without rearing. Concluding that sex does not affect exploratory behaviour from a single research model is risky, but the hypothesis is consistent with many rodent studies, including Ostaszewski and Pisula (1994) and Denti and Negroni (1975). In rabbits, the situation remains ambiguous because Pałka et al. (2015) found that males explored the open field more inten-

sively than females of the same species and that grooming frequency was similar in both sexes.

Pałka et al. (2015) also reported that a blue cube placed in the centre of the field aroused the interest of the tested animals. Females needed more time to approach the unfamiliar object and initially only males approached it. Males crossed more fields and were more interested in the arena walls, resulting in more rearing events. Thigmotaxis, i.e. peripheral movement as a passive-defensive response, was also consistent with the findings of Pałka et al. (2015). In the present study, however, selection of peripheral squares was more frequent in animals kept in the pasture system. Kowalska and Gugolek (2015) likewise described thigmotaxis in rabbits in the open field test. Increased exploration of the outer part of the field resulted in pasture-housed rabbits crossing the greatest number of fields, although the difference was not significant.

When interpreting the results, it should be remembered that a field was counted as crossed only when all four limbs of the animal were simultaneously within that square. Rabbits can certainly be regarded as timid animals, because their tolerance threshold for painful stimuli is higher than for stressful stimuli. Under stress, increased vigilance, accelerated breathing and freezing are often observed, and most animals displayed this type of response during the open field test. This is related to the release of noradrenaline and adrenaline from the adrenal medulla in response to stressors; prolonged exposure may therefore lead to blood-flow problems. Thumping is a common fear response in rabbits (Kowalska and Gugolek, 2007), but it was not observed in the present study.

Previous behavioural studies in rabbits have indicated that their behaviour is more similar to that of rats than to that of opossums. Rabbits show thigmotaxis, a passive-defensive behaviour manifested by selecting the outer fields of the arena in the open field test (Pałka et al., 2015). Another similarity is behaviour reflecting a conflict of drives: the trunk remains immobile while the head is extended forward to explore the surroundings. With repeated tests, rabbits may change their strategy for exploring the territory and often display a sniffing-locomotion-rearing sequence or a longer sequence ending in grooming (Kowalska and Gugolek, 2007). Budzyńska et al. (2018) reported that cattle also show fear responses manifested by intense observation and reluctance to explore a novel environment or contact a novel object or unfamiliar person, which can cause handling problems. In laboratory mice, Makino et al. (1991) observed immobility and stretching of the whole body as signs of emotional arousal associated with the novel open field environment.

Summary

Differences in the number of rearing events support the hypothesis that the housing system affects animal behaviour. The place in which animals live to some extent forces the development of specific mechanisms. Each housing system either restricts or enables particular ways of exploring the surroundings. Such constraints shape and consolidate certain patterns of exploratory behaviour. Rabbits kept on deep litter probably had the opportunity to dig through it (“burrow digging”), which is a natural behaviour of wild rabbits. They were therefore less stressed than rabbits in the other housing systems, which probably translated into a greater willingness to explore in the open field test. It should be remembered, however, that rabbits are timid animals and may therefore more readily adopt learned forms of behaviour. In addition, the open field test can be considered an extremely useful tool for assessing rabbit behaviour.

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