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Comparison of The Relative Brain Sizes and Learning Abilities of Animals of The Rodentia

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Abstract: Animals from the order rodentia are often used as experimental animals to study the fields of biology, medicine and animal husbandry, but are rarely used as objects of study to determine their position in evolutionary development and their role in the surrounding environment. One aspect of animal evolutionary development is learning behavior. Learning behavior in animals, especially those related to intelligence, is correlated with the relative size of the animal's brain. This research has examined the comparison of intelligence levels and relative brain size between animals, especially four species of animals from the Order Rodentia, namely mice (*Mus musculus*), mice (*Rattus assimilis*), hamsters (*Mesocricetus auratus*) and guinea pigs (*Cavia porcellus*). The learning ability of test animals was measured using the T-Maze procedure according to a complete group randomized experimental design. Data on the learning ability of the test animals were processed using ANOVA Model I and continued with the Orthogonal Contrast Test. The results of the research show that the average learning ability from highest to lowest is possessed by mice, hamsters, guinea pigs and mice. This learning ability data is in sync with data on relative brain size, namely the relative size of the largest brain in mice and the relative size of the smallest brain in mice. The learning ability of mice and rats, which both come from the same family, namely Disciples, is not significantly different from that of hamsters from another family, namely Cricetidae. Rats, mice and hamsters, which all come from the same suborder, namely Myomorpha, have much higher learning abilities compared to guinea pigs which come from another suborder, namely Hystricomorpha.

Keywords: Learning ability, relative brain size, rodentia.

Introduction

The world we live in is just a point in the vast universe. There is no consensus regarding the issue of how many years ago this life began. The oldest fossil that can be trusted is 500 million years old. Even though evolution must have occurred before that time, it turns out that fossils of organisms older than 500 million years have never been found. Until now, people are still studying the knowledge of when life began.

The study of the origins of living things and their development and manifestations in the form of similarities and differences is very helpful in classifying living things. On the other hand, knowledge about the classification of living creatures will later help in studying the phenomena of living creatures themselves, such as the structure and function of body parts as well as the diversity of living creatures as a whole and their behavior, especially in the animal world. According to Ambasciano (2017), one of the phenomena of living creatures, especially animals, which is related to their level of development in evolutionary history is learning behavior.

Animal behavior is a manifestation of the anatomy and physiology they have to deal with the environment in order to continue their life. The brain is a part of animal anatomy that is closely related to one type of behavior, namely learning behavior. According to Beauchamp, Yee et al., (2022), the level of brain development in animals in an evolutionary context can indicate their level of intelligence. The level of brain development of vertebrate animals (vertebrates) between animals from one class to another is clearly different so that their behavioral patterns and levels of intelligence are very easy to distinguish. However, these differences in brain anatomy become less clear when we compare them to animals in lower taxa of the class.

In general, comparisons of animal brains between families within an order are starting to become difficult to differentiate. Rodentia as one of the orders of the Mammalia class has quite high brain development. His cerebrum, whose function is related to learning ability has developed well (Dennis et al., 2021). Based on their skull structure, Rodentia is divided into three sub-orders, namely Sciuromorpha, Hystricomorpha, and Myomorpha. Mice, rats, hamsters and marmots are animals from the order Rodentia, which have almost the same brain structure. According to D'Elía et al., (2019), these animals occupy certain distinct positions in taxonomy. Mice and rats differ in species and even genus, namely mice (*Mus musculus*) from the genus *Mus* and rats (*Rattus assimilis*) from the genus *Rattus*. Mice and rats on the one hand and hamsters on the other hand are different families, namely mice and rats from the same family, namely Muridae, while hamsters from a different family, namely Cricetidae with the genus Mesocricetus. Mice, rats and hamsters on the one hand and marmots on the other hand differ in sub orders, namely mice, rats and hamsters from the sub order Myomorpha and marmot (*Cavia porcellus*) come from the sub order Hystricomorpha with the family Caviidae and the genus *Cavia*.

Environmental factors such as habitat, type of food, and type of competition can direct these animals to their level of intelligence or learning ability. The process is of course through natural selection, which produces selected brain microstructure anatomy that provides survival for generations of offspring. However, on the contrary, the special characteristics of both anatomy and physiology in these animals can also direct them to choose habitat, type of food and choose or develop certain types of competition, which is a manifestation of intelligence development (Bardos et al., 2024). The causal cycle behind the acquisition of this level of intelligence in these animals from where it started has not yet been determined. Moreover, the extent to which differences in these external factors can cause differences in animal intelligence levels cannot yet be known with certainty. However, we understand that mice, rats, hamsters and marmots have certain differences in taxonomic position. Departing from the problems mentioned above, this research was carried out with the aim of finding out the comparison of intelligence levels between mice, rats, hamsters and marmots. This study is also aimed at finding out the suitability of the comparison of intelligence levels and relative brain sizes of these rodents and their relationship with their position in animal taxonomy, especially in the order Rodentia.

Materials and Methods

Preparation of test animals

All species of test animals were selected that were still virgin, namely mice (*Mus musculus*) aged 5 weeks, rats (*Rattus assimilis*) aged 3 months, hamsters (*Mesocritetus auratus*) aged 2 months, and guinea pigs (*Cavia porcellus*) aged 3 months. From each population of test animal species, a sample of 6 individuals (replicates) was taken from Perum Biofarma Bandung. Before being used for experiments, the test animals were maintained and acclimatized in experimental environmental conditions for 7 days according to procedures modified from Bardos et al., (2024).

Experimental design

The learning ability of each test or treated animal species (Ti; i=1,2,3,4) was tested using the T-Maze procedure according to a completely randomized block design. A two-dimensional scheme of the T-Maze experiment used can be seen in Figure 1. The floor and side walls of the T-Maze are made from 12 mm plywood, while the roof is made from 6 mm reflective glass to form a maze like the letter T. The hallways in the T-Maze from the entry or start to the finish rooms have the same size, namely 12.5 cm wide and 19 cm high. The length of the hallways before branching is 50 cm. The length of each right and left branch aisle before the first turn is

25 cm. The length of the aisle from the first turn to the second turn, as well as the length of the aisle from the second turn to the finish room, is 87.5 cm respectively.

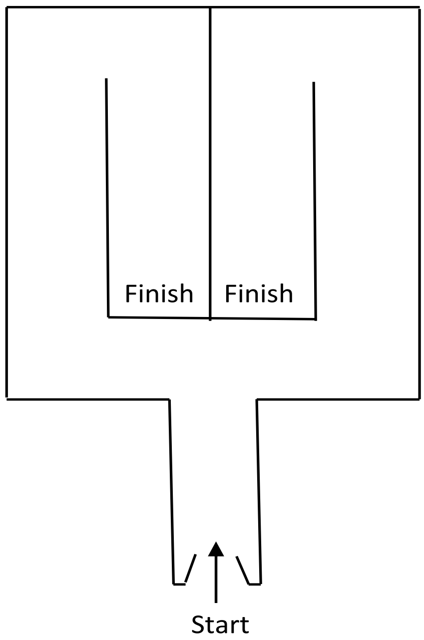

Figure 1. T-Maze for testing the learning ability of test rodents.

Learning ability experiments were carried out on 4 animal species or treatments (ri; i = 1, 2, 3, 4) and 6 individuals or 6 replications (n) were used for each animal species (ni; i = 1, 2, 3, 4, 5, 6). The use of 6 replications for each treatment fulfills the minimum sample size requirements for an experiment with 4 treatments according to the following formula (Suckow et al., 2012):

$$n = \frac{15}{r-1} - 1$$

Where
n = sample size (number of replications)
r = number of treatments (number of test animal species)

Each individual test animal was tested 10 times or rites. Each rite can be completed in a maximum of 15 minutes, so that in a day it can only be carried out on 3 individuals (replications) for each test animal species (treatment). Thus, to carry out all experiments on each species of test animal, it takes 2 different days or 2 experiment blocks (Bk; k = 1, 2). Thus, experiments on learning ability between animal species were conducted according to a randomized complete block experimental design. The design of the observation format can be seen in Table 1.

Table 1. Observation table format for the results of experiments on learning abilities in animals according to a completely randomized block design.

Block (B_i)	Treatment animal (Ti)			
	Mice (T_1)	Rat (T_2)	Hamster (T_3)	Marmot (T_4)
B_1	$B_1T_1n_i$	$B_1T_2n_i$	$B_1T_3n_i$	$B_1T_4n_i$
	$B_1T_1n_i$	$B_1T_2n_i$	$B_1T_3n_i$	$B_1T_4n_i$
	$B_1T_1n_i$	$B_1T_2n_i$	$B_1T_3n_i$	$B_1T_4n_i$
Subtotal				
B_2	$B_2T_1n_i$	$B_2T_2n_i$	$B_2T_3n_i$	$B_2T_4n_i$
	$B_2T_1n_i$	$B_2T_2n_i$	$B_2T_3n_i$	$B_2T_4n_i$
	$B_2T_1n_i$	$B_2T_2n_i$	$B_2T_3n_i$	$B_2T_4n_i$
Total				
Average				

Information
n_i = i^{th} repetition; i = 1,2,3,4,5,6; Three repetitions in one block, distributed completely randomly in two blocks

Measurement of the learning ability of test animals

Before being used for experiments, each species of test animal was acclimatized in a cage with experimental conditions for 7 days according to a modified procedure from (Hurtubise & Howland, 2017). The feed used for experimental animals is the same as the feed previously used at Perum Biofarma Bandung, namely corn-fish pellets for mice, rats and hamsters and a mixture of kale plant pieces and fresh carrot tubers for marmots.

During acclimatization, one preliminary experiment was also carried out on one individual of each test animal species, according to the modified procedures of Emery (2017) and Hiroyuki et al., (2018). Preliminary experiments were carried out during the day on the food-seeking activity of test animals that had been previously starved (animals had not been fed for 6 hours) in the T-Maze. These preliminary

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experiments were carried out to ensure that the test animal could or was successful in finding its food at the end of one of the branches in the T-Maze by observing the smoothness and duration of time taken by the test animal from the start of entering the T-Maze until it found food in the final tunnel. (finish) inside the T-Maze. After finding food in the final aisle, the animal is allowed to eat the food until the animal feels full. After that, the animal was removed from the T-Maze and returned to the acclimatization cage.

It is strongly assumed that after the preliminary experiment, the test animals were impressed to get a reward if they chose the "correct" path branch (successfully obtained food), namely one of the tunnel branches which at the end contained food from the two branches created in the T-Maze. The variables observed were the number of transits (walking back towards the entrance) and the duration of time taken in one trial until successfully finding food in the final room of one of the branch tunnels in the T-Maze. After preliminary experiments, main experiments were then carried out to measure the learning ability of the test animals. The working steps are the same as in the preliminary experiment, according to the modified procedure from Emery (2017) and Hiroyuki et al., (2018), unless each experiment is carried out in 10 rounds for each individual test animal, the time duration for each test cycle is a maximum of 15 minutes, during which the test animal succeeds or fails in finding food in the final tunnel in the T-Maze.

After 15 minutes of experimenting in the T-Maze, the test animals were removed from the T-Maze through a roof that could be opened or closed, then put back into the T-Maze through the entrance (start) for the next round. For each individual (repetition) the test animals was tested in 10 rounds. The variable observed in each round is the success of finding food, namely giving a score of 1 if successful and a score of 0 if unsuccessful, and counting the number of transits (walking back towards the entrance) made by individual test animals. The format of the observation table for the results of the learning ability experiment for each individual test animal or each replication can be seen in Table 2.

Table 2. Observation format of learning ability for each repetition (individual) of test animals

Animal treatmen (Ti)		Sample or repetition number: …… (1,2,3, … or 6) Experiment block: … (1 or 2)	
RN	X1	X2	Y $\frac{(X1 + X2) - X2}{(X1 + X2)} \times 100$
1	……	…….	……………
2	……	…….	……………
3	……	…….	……………
…	……	…….	……………
10	……	…….	……………
Average			……………

Information:
Ti; i = 1,2,3,4
T1 = mice; T2 = rat; T3 = hamster; T4 = marmot
NR = Round number
X1 = Success score for choosing a branch (1 if successful, 0 if unsuccessful)
X2 = Number of transits (moving back towards the entrance or start)
Y = Learning ability score

After all individual animals (replications) were tested, the data on the learning abilities of the test animals were compiled in a table in the following format (Table 3).

Table 3. Format of experimental observation results on the learning ability of four rodent species

Blok	No. Replican	Animal treatment (Ti)			
		T1	T2	T3	T4
1	4				
	1				
	3				
	Subtotal				
2	6				
	2				
	5				
	Subtotal				
	Total				
	Average				

Brain measurements of test rodents

After their learning abilities were measured, all test animals were euthanized and their brains dissected, using a modified procedure from Beauchamp, Yee, Darwin, Raznahan, Mars & Lerch (2022), Dennis *et al.* (2021) and Welniak-Kaminska *et al*. (2019). The weight of the body, whole brain and cerebrum of each individual test animal was measured respectively. The relative size of the brain of each test animal was then calculated, namely the weight percentage of the whole brain to the body, the weight percentage of the serebrum to the body and the weight percentage of the of the serebrum to the whole brain.

Data analysis

Data on the learning abilities of mice, rats, hamsters and marmots were tested for differences using ANOVA Model I according to a completely randomized block experimental design. There are 4 species of treated animals, each with a sample size of 6 animals (replications).

After ANOVA, further comparison tests were carried out using the orthogonal contrast method. This orthogonal contrast test is carried out to test one-way average differences between treatments and at the same time test one-way differences or contrasts between previously determined treatment groups. Grouping of treatments (test animal species) is determined hierarchically (orthogonally) based on their position in animal taxonomy, especially in the order Rodentia. The comparison or contrast formula is determined as follows (Figure 2):

$C_1 =$	T_1	$- T_2$
$C_2 =$	$T_1 + T_2$	$- 2T_3$
$C_3 =$	$T_1 + T_2 + 2T_3$	$- 4T_4$

Figure 2. Design of orthogonal contrasts of learning abilities between animals in the Order Rodentia
Information:
C_1 = contrast between T_1 and T_2
C_2 = contrast between $T_1 + T_2$ and $2T_3$
C_3 = contrast between $T1 + T_2 + 2T_3$ and $4T_4$
T_1 = Treatment 1 (mice)
T_2 = Treatment 2 (rats)
T_3 = Treatment 3 (hamsters)
T_4 = Treatment 4 (marmots)

Results and Discussion

Feasibility and significance of data on the learning ability of test animals

In this study, two types of data were obtained, namely the average learning ability score of the test animals as the main data and the average relative size of the brains of the test animals as additional data. Measurements of the learning abilities of the four test animals were each carried out on 6 animals (6 replicants). Because the samples of all species test animals were taken independently from the population at Perum Biofarma Bandung, the average comparison test between treatments was carried out using ANOVA Model I (independent model).

Experiments can only be carried out during the day and can only be completed 3 replications a day, so to complete 6 replications it takes 2 different days or 2 blocks of experiment. To reduce the influence of this block, the six replicates were evenly distributed randomly into two blocks. For this reason, the experiments were carried out using a completely randomized block design.

To test the average differences between treatments using ANOVA Model I, first testing the normality of the data for each treatment using the Lilliefors test and testing the homogeneity of the combined variance of all treatments using the Bartlett test. The results of the normality and homogeneity tests show that the learning ability data for mice, rats, hamsters and guinea pigs each come from a normally distributed population and the combined variance of the learning ability data for all species of test animals mentioned above is homogeneous. Thus, the learning ability data for the four species of test animals is suitable for further processing using ANOVA Model I.

The results of ANOVA Model I according to a completely randomized block design show that the experiment block factor did not have a significant effect on the treatment average, where for the block factor, the Fcount value (0.3017) was lower than the Fcrit value (2.9013), which means that the average between blocks is not significantly different, while for the treatment factor, the Fcount value (0.3017) was higher than the Fcrit value (2.9013), which means that the average between treatments is significantly different (Table 4).

Tabel 4. Summary of ANOVA results on the learning abilities of 4 rodent species

Summary	*n*	*Sum*	*Average*	*Variance*
Mice	2	109,43	54,71 (a)	0,3788
Rat	2	85,83	42,91 (d)	0,5669
Hamster	2	97,61	48,81 (b)	0,0139
Marmot	2	91,80	45,90 (c)	0,0084
Block 1	4	192,28	48,07 (a)	21,7867
Block 2	4	192,38	48,10 (a)	29,1960

Source of Variation	SS	df	MS	F	P-value	F crit
Treatments	151,9819	3	50,6606	157,2202	0,0009	9,2766
Blocks	0,0013	1	0,0013	0,004	0,9534	10,128
Error	0,9667	3	0,3222			
Total	152,9498	7				

Direction of comparison of learning abilities between test animals

The results of further ANOVA using the orthogonal contrast method showed that the average learning ability between test animals was significantly different. The average learning ability from highest to lowest was respectively possessed by mice, hamsters, marmots and rats (Figure 3).

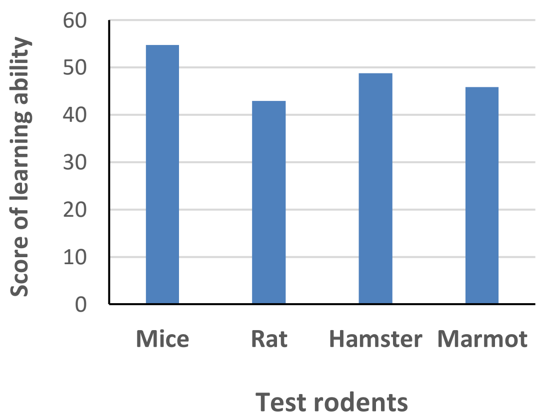

Figure 3. Learning ability scores in rodents (average of 6 replicates)

The follow-up ANOVA also produces 3 comparisons or contrasts, namely C1, C2 and C3. In the first contrast (C1), the average learning ability of mice was significantly higher than that of rats. These two rodent species come from the same family, namely Muridae. For this reason, in visual comparisons, as shown in Figure 6, the rats with the lowest learning ability were positioned closest to the mice with the highest learning ability.

In the second contrast (C2), the average amount of learning ability of mice and rats on the one hand (family Muridae) was not significantly different from the average amount of learning ability of hamsters on the other hand, which comes from the family Cricetidae. In the third contrast (C3), mice, rats and hamsters on the one hand, all three from the same sub-order, namely Myomorpha, have an average learning ability that was significantly higher than that of marmots on the other hand, which comes from the suborder Hystricomorpha (Table 5, Figure 4).

Table 5. Comparison of learning abilities between animals in the Rodentia order

	CT1	CT2	Information
C1	54.75	42.97	CT1>CT2 significantly
C2	97.75	97.48	CT1 and CT2 are not significantly different
C3	195.20	183.56	CT1>CT2 significantly

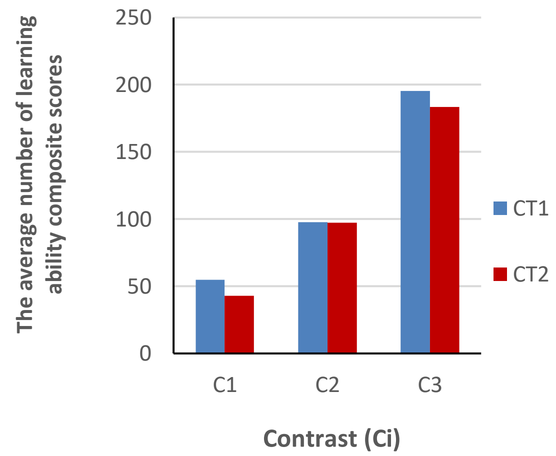

Figure 4. The contrast of learning abilities between animals in the order Rodentia

CT = Combined treatments
C1: Contrast CT1 (mice) with CT2 (rats), where CT1>CT2, sigficantly
C2: Contrast CT1 (mice + rat) with CT2 (hamster), CT1 was not significantly different from CT2
C3: Contrast CT1 (mice + rat + hamster) with CT2 (marmots), where TG1>CT2

Mice have a higher learning ability than hamsters. There are several reasons to explain this. According to Bolhuis (2015), Spear, Miller & Jagielo (2020), Zentall, Wasserman, Lazareva, Thompson & Rottermann (2008) and Vorhees & Williams (2014), rodent animals from the family of Muridae, for example mice, began to appear in the Myosin era (10-15 million years ago) which was an evolutionary development of their ancestors, namely the hamster "family" or Cricetidae. However, the development of members of the Muridae family does not always produce a higher level of intelligence than their ancestral member. Rats, for example, which are members of the Muridae family, which in this research were proven to actually have a lower level of learning ability than hamsters, which are members of their ancestral family, namely Cricetidae.

Mice and hamsters on the one hand from the Myomorpha suborder have higher learning abilities than guinea pigs on the other hand from the Hystricomorpha suborder. This may have something to do with the position or level of evolution of marmot which is more primitive than the other two species mentioned previously. The masseter muscles of rodents from the Hystricomorpha suborder, including marmot, show a more primitive attachment (Welniak-Kaminska, Friedoworicz, Orzel, Bogorodzki, Modlinska, Stryjek, Chrzanowska, Pisula & Grieb, 2019; Wood, 2015).

Relative brain size and learning ability of test animals

It has become common knowledge that the level of progress in animal evolution can be seen from the level of progress in their brain structure. The level of development of an animal's brain structure is closely related to the relative surface area of all brain nerve tissue to the total surface area of the animal's body. The level of progress in animal evolution, especially related to the level of intelligence, can be seen in the level of progress in brain structure, especially the part of the brain that is related to intelligence functions, namely the cerebrum (Welniak-Kaminska et al., 2019; Rukmana, Fadil, Gafur, Isnaeini, Azizah & Safira, 2023; Spear *et al*., 2020).

However, to determine the level of progress of the brain structure as a whole or specifically the cerebrum by measuring the surface area of the total neural network within it is very difficult and there is no feasible method to date (Dennis *et al*., 2021). So in this study, observations of the relative size of the animal's brain were carried out on another variable that substitutes for brain area, namely the relative weight of the brain of all the test animals observed. The measurement results show that the relative size of the brain, i.e. the percentage of weight of the cerebrum to the body from highest to lowest, was owned by mice, hamsters, marmots and rats respectively (Figure 5).

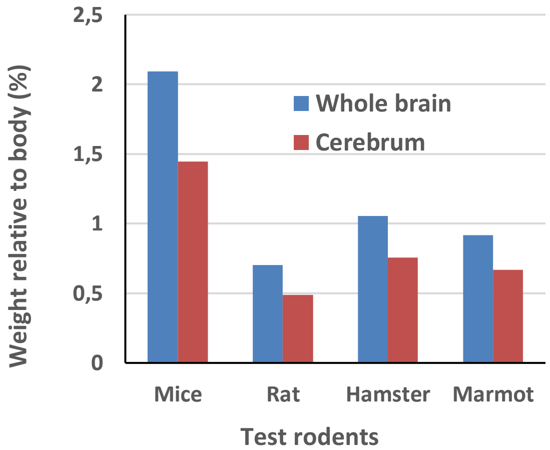

Figure 5. Relative weight of the brain to the body in rodents

The order of relative brain size of the animals observed in this study was synchronous with the order of their learning ability levels. These results strengthen the notion that the relative size of the brain, especially the cerebrum, greatly determines the level of intelligence in animals, including animals from the Order Rodentia.

Conclusion

The ability to learn from the highest to the lowest was respectively possessed by mice (Mus musculus), hamsters (Mesocricetus auratus), marmots (Cavia porcellus), and rats (Rattus assimilis). The learning ability of mice and rats on one side of the Muridae family was not significantly different from the learning ability of hamsters on the other side of the Cricetidae family. The learning ability of mice, rats and hamsters on the one hand from the Myomorpha suborder was significantly higher than that of marmots on the other hand from the Hystricomorpha suborder. The relative weight of the brain, namely the percentage of weight of the cerebrum to the body of mice, rats, hamsters and marmots, was 1.45, 0.49, 076 and 0.67% respectively.

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