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Environmental and dam effects on cannibalism in Wistar rat litters

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ABSTRACT. The factors related to cannibalistic behavior of dams in a Wistar rat colony are identified and analyzed. The maternal genetic effects were tested as a random effect by the method of generalized linear models. The season at parturition, the dam´s age at parturition and the density of the room at parturition were tested as fixed effects, whereas the litter size at birth was tested as a co-variable. The genetic effect of the dam was significant for the number of cannibalized pups. Although the season at parturition, the dam´s age and room density on the day of parturition were not individually significant ($p > 0.05$), most of the interactions between the variation sources were significant ($p < 0.05$). Cannibalism occurred mostly in dams aged over 241 days, with parturition during spring. So that occurrences of cannibalism could be avoided, dams with the smallest number of cannibalized pups should be selected, coupled to dams younger than 241 days, breeding during spring. The above strategies may reduce the number of couples in the vivarium and increase their production efficiency.

Keywords: laboratory animals, maternal behavior, infanticide, rodents.

Efeito do ambiente e da matriz sobre o canibalismo em filhotes de ratos Wistar

RESUMO. O objetivo deste estudo foi identificar os fatores relacionados ao comportamento de canibalismo das fêmeas em uma colônia de ratos Wistar. Os efeitos genéticos maternos foram testados como efeito aleatório usando o método dos modelos lineares generalizados. A estação do parto, a idade da fêmea ao parto e a densidade da sala ao parto foram testados como efeitos fixos e o tamanho da ninhada ao nascimento foi testado como covariável. O efeito genético da matriz foi significativo para o número de filhotes canibalizados. A estação do ano ao parto, a idade da matriz e a densidade da sala no dia do parto quando testados separadamente não foram significativos ($p > 0,05$), porém, a maioria das interações entre estas fontes de variação foram significativas ($p < 0,05$). O canibalismo ocorreu em maior frequência em fêmeas com idade superior a 241 dias durante a primavera. Para evitar o canibalismo na população estudada, recomenda-se a seleção de fêmeas com menor número de filhotes canibalizados e a maior utilização de matrizes com idade inferior a 241 dias durante a primavera. Estas estratégias podem promover a redução do número de casais necessários no biotério e aumentar sua eficiência reprodutiva.

Palavras-chave: animais de laboratório, comportamento materno, infanticídio, roedores.

Introduction

Increasing production rates of laboratory animals in Brazil and worldwide are necessary because of the great expansion and intensification of research performed in a wide variety of disciplines, including biology and human and veterinary medicine. In addition, the 3 Rs principle (replacement, reduction, and refinement) recommends that the number of laboratory animals should be reduced. Breeding practices adopted in vivariums should be evaluated and adapted to increase the production efficiency and the quality of the product offered to researchers. The above will ensure that animals respond satisfactorily in experiments (FORNI, 2007).

Certain laboratory rat strains are used in the exploration of genetic variability and the identification of phenotypes. In the case of genetically heterogeneous rodent lines (also called outbred lines), which include the Wistar rat line, the selection may be used in favor of traits of interest in animal breeding (FERREIRA et al., 2005). The practice is possible because phenotypic and genetic variability exists for traits of interest in Wistar populations and these traits include those related to productivity and behavior of the dams. The number of animals weaned per litter is one of the most important economic variables in vivariums and several factors may interfere with this productivity, particularly maternal ability and behavior (PÉREZ-

LASO et al., 2008). Therefore, dams with better reproductive performance and a greater number of weaned animals per litter should be selected since the variable is highly relevant in Wistar rats.

The post-parturition behavior of these dams must be monitored to verify a possible decrease in the number of weaned offspring, caused by the dam´s cannibalism of its pups, also known as infanticide. Maternal behavior corresponds to a series of stereotypical actions, such as posture during the suckling of their young, cleaning of pups, nest preparation, grouping of the litter and defense of the offspring against attackers (BONSIGNORE et al., 2006; PÉREZ-LASO et al., 2008). This behavior, influenced by genetic and environmental factors and by sensory stimuli of the offspring, is maintained while nursing but tends to decline during lactation (LONSTEIN; GAMMIE, 2002; BONSIGNORE et al., 2006). Infanticide of offspring has been observed in rodent dams and may occur when the dams do not exhibit the expected perinatal characteristics. Malformed and weak pups, as well as those with low body temperature and little vocalization, are identified as the pups with low survival possibilities and, therefore, prone to be cannibalized by their dams (D'AMATO et al., 2005). In other words, infanticide may be a manifestation of healthy offspring protection, resulting in the elimination of pups that would not survive. Cannibalism of pups may also be a survival strategy for adults during periods of food restriction or population regulation (GETTO et al., 2005).

Mutations in the rodents´ gene encoding the prolactin receptor have been reported and may trigger disorders in maternal behavior. Additionally, mutations in genes encoding estrogen and vitamin D receptors lead to abnormal behaviors, such as an increase in cannibalism which interferes directly with the dam´s productivity (CHAMPAGNE, 2008). Further, cannibalism plays a role in improving the skeletal conditions for females at weaning; the cannibalistic females improve the mineral content of their femurs by reducing the overall demand of suckling (HOOD, 2012) and thiamine deficiency may induce perinatal killing responses (BÂ, 2013).

Therefore, investigation on the maternal behaviors of Wistar rats, especially those related to the cannibalism of pups, is necessary to select dams that develop behaviors within the expected standards. Possible sources of variation that may affect cannibalistic behaviors in various species include the season at parturition, animal density per room and the age of dams at the time of parturition (KORPELA et al., 2011).

On the other hand, since few studies have been undertaken on this subject or on the cannibalism of pups, investigations may favor strategies to help select successful Wistar breeders in vivariums. The identification of factors that alter this behavior will enhance the selection and maintenance of reproduction by dams with normal maternal behavior. By increasing dam reproduction rates, the production system is optimized and becomes more efficient.

Current study investigates whether the dam, the season at parturition, the dam's age at parturition or the density of animals in the room on the day of parturition are related to cannibalism exhibited by Wistar dams at the Central Vivarium of the Federal University of São João del Rei, São João del Rei, Minas Gerais, Brazil.

Material and methods

Current study was approved by the Ethics Committee on the Use of Animal at the Federal University of São João del-Rei, São João del-Rei, Minas Gerais, Brazil (Protocol n. 23/2010).

Collection of data

Current assay used 144 monogamous rats paired from the Wistar Hannover line (*Rattus norvegicus*) and kept at the Central Vivarium of the Federal University of São João del Rei, which operates under conventional conditions but without strict sanitary barriers. The rats were maintained in an environment at a controlled temperature (between 18 and 22°C) and a 12-h light cycle. Water and special feed for laboratory rodents were offered *ad libitum* during the experimental period.

The rats that have been paired were kept together during all the experimental period, in an exclusive room for breeders. The cage for each pair measured 414 x 344 x 168 mm and beds and nests were made of clean wood shavings replaced every two days.

Data were collected between August 2010 and January 2011. In addition to the rat colony's pedigree, the data on animal breeding included the date of each dam's birth, the age of dams at the time of parturition, the date of parturition, the number of pups born per parturition per dam, and the density of the room where the dams were kept on the day of parturition.

All births occurring during each couple´s productive lifetime in the vivarium were reported. The pups were counted after each parturition and data recorded on specific forms. Counting occurred three times per week until the litter was weaned. Missing pups and those found dead with mutilations were considered cannibalized. Based on the difference between the pups born and the pups weaned, the variable in current study, the number of missing pups (MIS) was generated and counted per pair, at each parturition, from birth of pups until

weaning. Data of 697 parturitions were registered; 7,202 pups were born and 358 were cannibalized.

Data analysis

Data were processed in the Laboratory of Experimental and Computational Neuroscience in the Department of Biosystem Engineering at the Federal University of São João del-Rei, São João del-Rei, Minas Gerias, Brazil. Descriptive statistics were also calculated (number of observations, mean, standard deviation, median, and minimum and maximum rates) for MIS with Statistical Analysis System® (SAS, 2008). The sources of variation tested comprised randomized and fixed effects. The random effect was the effect of the dam (DAM) or the dam´s individual genetic contributions. The fixed effects were: season at parturition (SEA), or the season at the time of parturition, based on the date of the parturition; class of room density (DEN), or the number of animals in the room on the date of parturition (these effects were distributed into 3 classes, as described in Table 1); and class of age of the dam (AGE), or the age of the dam, in days, recorded on the parturition date (these effects were distributed into 4 classes, as described in Table 1).

Table 1. Distribution of levels of the fixed effects tested on the variable MIS.

	Classes			
	1	2	3	4
SEA	Spring	Summer	Fall	Winter
DEN	50 to 100	101 to 150	Over 151	-
AGE	60 to 120	121 to 180	181 to 240	Over 241

MIS = number of missing pups; SEA = season at parturition; DEN = room density at parturition; AGE = age of the dam at parturition.

The distribution of levels (classes) for the fixed effects tested for the MIS is given in Table 1, and the frequencies of the data for these levels are given in Table 2.

Table 2. Frequencies of the variable MIS for the fixed effect levels.

Fixed effect	Class[a]			
	1	2	3	4
SEA	183	248	153	113
DEN	303	251	143	-
AGE	171	217	175	124

MIS = number of missing pups; SEA = season at parturition; DEN = room density at parturition.

In addition to the sources of variation already described, the litter size at birth per dam (LIT) was tested as a co-variable for MIS.

The interactions among the possible sources of variation, the co-variable and the variable MIS were also tested. Further, the correlation between MIS and LIT was estimated.

Analyses evaluating the fixed effects, the random effect and the co-variable were performed by the method of generalized linear models with Statistical Analysis System® (SAS, 2008), with statistical significance level at 5%. Tukey-Kramer´s test compared the averages of the significant fixed effects; the statistical significance level was also set at 5%.

Results and discussion

Table 3 shows the descriptive statistics for MIS and LIT, whereas Table 4 demonstrates p-rates for the fixed and random effects, the co-variable and the interactions tested for MIS.

Table 3. Mean (M), standard deviation (SD), median (MED) and the minimum (MIN) and maximum (MAX) rates for the variable MIS and the co-variable LIT.

	M	SD	MED	MIN	MAX
MIS	0.51	1.11	0	0	7
LIT	10.33	3.37	11	1	18

MIS = number of missing pups; LIT = litter size at birth.

Table 4. p-rates for fixed and random effects, co-variable and interactions tested on MIS.

Source of Variation	p-rate
Fixed effects	
SEA	0.307
AGE	0.062
DEN	0.190
Random effect	
DAM	$< 1 \times 10^{-4}$
Co-variable	
LIT	$< 1 \times 10^{-4}$
Interactions	
SEA*AGE	8×10^{-4}
AGE*DEN	0.604
LIT*AGE	0.025
LIT*DEN	0.016
LIT*SEA	0.060
SEA*DEN	_

MIS = number of missing pups; SEA = season at parturition; AGE = age of dam at parturition; DEN = room density at parturition; DAM = random effect of dam; LIT = litter size at birth.

Since there was no significant difference ($p > 0.05$) when the fixed effects were tested individually over the variable MIS, the season at parturition ($p = 0.307$) and the age at parturition ($p = 0.062$) did not affect individually the number of cannibalized pups. Room density too did not affect this behavior in the Wistar line ($p = 0.190$). However, the effect of DAM was significant for MIS ($p < 1 \times 10^{-4}$) and indicated the dam´s individual genetic contributions. Therefore females that exhibit this cannibalistic behavior may genetically transmit it to their progeny.

The interaction between AGE and SEA was significant ($p = 8 \times 10^{-4}$) for the variable MIS and indicated that these factors, when jointly evaluated, may affect the cannibalistic behavior in this line. Females over 241 days old with parturition during the spring differed statistically from the others (Table 5). The interaction between AGE and DEN was not significant for

the variable MIS (p = 0.604). In other words, these interconnected factors were not able to explain the cannibalistic behavior of the dams.

Table 5. Comparison of the average of the variable MIS by the Tukey-Kramer´s test for the interaction between season at parturition and age of the dam.

		AGE			
		1	2	3	4
SEA	1	0.312[a]	0.835[a]	0.315[a]	1.883[a]
	2	0.361[a]	0.173[a]	0.047[a]	0.326[b]
	3	0.787[a]	0.976[a]	0.615[a]	0.637[b]
	4	0.926[a]	1.025[a]	0.784[a]	0.946[ab]

MIS = number of missing pups; AGE = age of the dam; SEA = season at parturition. Different letters in the same column differ statistically (p > 0.05).

Since the interaction between LIT and AGE was significant (p = 0.025) for the variable MIS, the cannibalistic behavior of dams may depend on the dam´s age and on the number of pups born. Similarly, the interaction between LIT and DEN proved to be significant (p = 0.016).

The effect of LIT, within an interaction with SEA, was not significant (p = 0.060) and suggested that these factors, when associated with each other, failed to affect MIS.

The interaction between SEA and DEN resulted in an entanglement with the other sources of variation evaluated; this rendered impossible their inclusion in the analyses. The effect of this interaction was thus excluded from the model.

The correlation obtained between LIT and MIS was positive and low (0.23), albeit significant ($p < 1 \times 10^{-4}$); this result indicated that an association existed between the two traits.

Getto et al. (2005) underscored that a cannibalistic behavior was a means to regulate population. In fact, several recent studies reported that the density of the room could affect the cannibalistic behavior of *Myodes glareolus* (KORPELA et al., 2011), a wild rodent native to Finland that has been maintained under controlled conditions. A high density of rodents increases the index of cannibalism for the species. However, the above result has not been verified in current study, perhaps due to a different rodent line and/or other factors involved with the cannibalistic behavior of the dams in this population.

The significant effect that an individual dam had over the variable MIS demonstrated that females characterized by this kind of behavior could genetically transmit it to their offspring. A study of heritability for this variable may demonstrate with great reliability the degree of heredity this behavior possesses, based on the estimation of their variance components (VISSCHER et al., 2008). While some authors claim that cannibalism may occur due to factors such as density (KORPELA et al., 2011) and the frequency of bed changes (BURN et al., 2008), it appears that hormonal changes or fundamental changes from a genetic source may trigger aggressiveness in dams (LONSTEIN; GAMMIE, 2002). Thus, animal breeding should select dams that are not subject to this behavioral deviation and thus enhance the control of such behavioral disorders in new generations. Consequently, a lower number of dams will be necessary to produce the same number of pups because the selected females will have higher reproduction efficiency, resulting in a greater number of weaned pups and thereby reducing the number of pairs necessary in vivariums.

Females over 241 days old experiencing parturition during the spring differed statistically from the other dams under analysis. This fact may be due to climatic changes during the spring season, particularly changes in temperature and in the rainfall regime, which may cause slight fluctuations in the internal environment of the vivarium. These climatic changes would result in an increase in heat and humidity, which gradually intensify during the season (INPE, 2013).

Physical environmental factors, such as temperature, relative humidity, ventilation, photoperiod, noise, gases and particulate substances, may influence the biological responses of rodents (BESCH, 1980). Based on the above mentioned results, it may be concluded that older dams have a greater difficulty in adapting to this new condition, with the subsequent increase in their cannibalistic behavior at this time of year. Therefore, females less than 241 days old should be used mostly in the spring since at this period they have greater production efficiency, resulting in a greater number of weaned pups and reducing the number of dams necessary in the vivarium during the spring.

The significant interaction between the litter size at birth and the dam´s age demonstrates that the cannibalistic behavior of the dam may be affected by age and the number of pups born. Thus, it is possible that older dams have less maternal ability when faced with a higher number of pups.

Similarly, the significant interaction between the litter size at birth and room density may have occurred because cannibalism may be considered a population regulator (ANDREASSEN; GUNDERSEN, 2006). Depending on the number of animals born and the density of animals in the room, dams may exhibit cannibalistic behavior to regulate population size.

The low and positive correlation observed between the LIT and the MIS suggests that an

association exists between the two traits. It may be thus expected that the greater the number of animals born per parturition, the greater the chance that cannibalism will occur. Although only a low association degree existed between the two traits, there was a significant effect of the LIT co-variable over the MIS evaluated in the model, as described above.

Conclusion

It may be recommended that the Central Vivarium would select Wistar dams that are less likely to cannibalize their pups so that the occurrence of this behavior could be controlled in future generations.

An increase in the number of dams under 241 days old during the spring is recommended to further reduce the occurrence of cannibalism in the animal house.

The density of animals associated to the number of pups born per litter may influence the number of cannibalized pups. Further studies should be undertaken for more in-depth knowledge and data on the effects of humidity and animal density in vivariums housing Wistar rats.

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