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Nest-building behaviour in male rats from three inbred strains: BN/HsdCpb, BDIX/Or1Ico and LEW/Mol

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Abstract

Three inbred strains of male laboratory rat (BN/HsdCpb, BDIX/Or1Ico and LEW/Mol) were provided with nest boxes and nest materials, and were observed for nest-building activity. After 7 days, each cage and nest box was examined. Each nest was weighed and scored for complexity, and returned to the cage. This was repeated after a further 7 days and the nest removed completely. This routine was repeated three times. All three strains of rat built nests inside the nest box and showed the same stages of construction. There was a significant increase in nest complexity between day 7 and day 14 in the strains BN and BDIX. Furthermore, BDIX rats used significantly more material for the nests, compared with the BN and LEW rats. In a second experiment, using the same rats, nest material was offered in four different ways. LEW rats used nesting material irrespective of where it was placed; BN rats only used straw placed on the top of the cage when no alternative was provided in the cage, and would not use the nest box roof when it was covered with bedding; whereas BDIX rats would only use nest material placed within the cage and would not use straw placed on the cage lid. BN rats also used nest material to cover the entrance to the nest box, a practice not carried out by the other strains. This study demonstrates that these rat strains have retained their natural nest-building behaviour. We suggest that the correct stimuli must be provided in order for this behaviour to be exhibited; in addition, the way in which the nest materials are provided must be in accordance with strain-specific characteristics.

Keywords: *animal welfare, behaviour, enrichment, housing, nest, rats*

Introduction

The natural habitat of the Norway rat (*Rattus norvegicus*) is scrubby grassland where they establish colonies that are divided into smaller subgroups. The rats construct extensive networks of tunnels with several burrows separated by small tunnel segments. If the tunnel system becomes too crowded, a pregnant female or a stray male will leave and establish its own burrow either in connection with the colony it comes from or elsewhere.

The burrows are used as food caches and nest sites, and for social activities, such as playing, resting and grooming. The entrance holes to the burrows are often covered by plant material to conceal the entrance to the burrow and to prevent drafts. The nests can be shared by several adult rats of both sexes and have the same basic features: a depression in the ground of the burrow lined with coarse plant material on which grass, straw or leaves are placed. Fresh nest material is collected by the individuals of the group to prevent the nest from becoming worn. The nest can be cup- or bowl-shaped with a circular opening, and may be covered by a roof to resemble a hut (Calhoun 1962).

Nest-building is an innate behaviour, which is also performed by rats reared in isolation (Kinder 1927; Eibl-Eibesfeldt 1961). It consists of a number of fixed action patterns and skills that have to be stimulated for nesting to be successful. Factors that cause the innate releasing mechanisms for the fixed action patterns differ in strength and origin depending on the physiological state and maturation of the animal, and can be modulated by releasing mechanisms of other fixed action patterns (Ewert 1980). For example, injections of sex hormone have a strong positive influence on nesting behaviour in both female and male rats (Bermant & Davidson 1974; Ewert 1980). Eibl-Eibesfeldt (1961) describes the action patterns of the rat's nesting behaviour: phase one, digging the burrow; and phase two, nest construction. The second phase can be subdivided into foraging for material and nest-building.

Laboratory rats can exhibit all the behaviours of their wild ancestors and can become feral (Berdoy 2003). It has been observed that laboratory rats do not display much purposeful nest-building activity (except when females are approaching parturition) even when they are provided with nest material, and that their sleeping sites usually have little structure (Lawlor 2002; Van Loo & Baumans 2004). However, in a previous study, it was found that nest-building behaviour in Sprague-Dawley (SD) rats (of the

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Table 1 Cleaning and enrichment routines.

Weekday	Experiments I and II	Experiment I				
		Standard set-up enrichment				
	Clean cage	Nest box	Bedding on cage floor	Wood wool outside nest box on cage floor	Gnawing stick	Straw outside nest box on cage floor
Mon	x	x	200 g	20 g		20 g
Tue		x				
Wed		x				
Thu		x	200 g		1	20 g
Fri		x				
Sat		x				
Sun		x				
Mon		x	200 g	20 g		
Tue		x				
Wed		x				
Thu		x	200 g		1	20 g
Fri		x				
Sat		x				
Sun		x				
Mon	x	x	200 g	20 g		20 g

Mol: SPRD Han strain) is not limited to females; males of this stock also exhibit nest-building activity (Jegstrup & Ritskes-Hoitinga 2004).

Inbred strains of rat are characterised by little or no genetic differentiation between individuals of the same strain. Laboratory rats are not selected for their nest-building ability and as their survival and reproduction is not dependent on this ability, conservation of the underlying genes cannot be presumed. Under no selective constraints, a loss of functionality in the underlying genes could provide an explanation for the observations by Lawlor (2002) and Van Loo and Baumans (2004) regarding low levels of nest-building activity. However, an alternative explanation would be the lack of appropriate stimuli to trigger nest-building behaviour. Before phase one — the digging of the burrow (Eibl-Eibesfeldt 1961) — is triggered, the rat must go through another phase: the finding of a suitable site to establish the burrow, which must be peaceful to be a success. This could explain the high demand for nest boxes in the operant studies of Townsend (1997) and Manser *et al* (1998a) and why a closed, dark type of nest box was preferred (Manser *et al* 1998b). If the animal is successful in finding the object of its search (the appetitive behaviour), motivation for the next behavioural phase (the consummatory act [Manning 1979]), in this case the construction of the nest, is increased.

In this study, a large nest box was provided in the cage as an object for the rats' appetitive behaviour in the hope that it would prove a suitable site for nesting, as observed in the previous study of SD rats (Jegstrup & Ritskes-Hoitinga 2004). In addition to wood wool, straw was also supplied in the cages as the rats were not able to build nests similar to those seen in nature with only wood wool or paper (personal observation). We tested the hypothesis that males also retain nest-building behaviour, if the proper environmental tools are provided. Behavioural differences in nest-building activity were compared using three inbred strains of rat.

Materials and methods

This study consisted of two experiments carried out in succession using the same rats, with three groups of two pair-housed animals per strain.

Experiment 1

Male rats from three inbred laboratory strains were obtained at 10 weeks of age: BN/HsdCpb (obtained from Harlan, Horst, The Netherlands); BDIX/OrlIco (obtained from Charles River-Iffa Credo, L'Abresle Cedex, France); and LEW/Mol (obtained from Taconic M&B, Ry, Denmark). These three strains are hereafter referred to simply as BN, BDIX and LEW, respectively. The rats were given two weeks to become acclimatised before the start of Experiment I. Experiment I lasted 42 days. Prior to the start of the experiment, the rats had not had access to nest boxes or straw. They were housed in pairs of the same strain in Macrolon™ IV cages, 48 × 38 × 20 cm (length × width × height), of transparent polycarbonated plastic with a stainless steel wire roof. Aspen wood chips were provided as bedding (Tapvei® Oy: Kaavi, Finland).

Each cage was equipped with a nest box in the middle of the cage. The nest box measured 28.5 × 20.5 × 12.5 cm (length × width × height) internally, similar to the average size of a burrow in the wild, and was made of wood, 1 cm thick, held together with wooden nails. Beech wood was used, as the durability of aspen was found to be unsatisfactory in an earlier pilot study (unpublished data). The box had two rounded entrances/exits (6 cm high at the highest point and 6 cm wide; see Figure 1). Aspen wood wool, aspen gnawing sticks (Tapvei® Oy: Kaavi, Finland), and autoclaved wheat straw were placed outside the box. Straw is not normally used in the laboratory setting, but we chose to use it in this

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Table 2 Score sheet for the stages of nest-building activity.

Advancing stages →	1. Bedding gone	Bedding material is gone from the specific nest site.
	Use of nest material:	
	2. Without structure	Nest material has been placed in the nest site.
	3. Mat	Nest material has been placed at the nest site and made into a flat mat (3–5 cm thick is not uncommon).
	Cup	The nest is built up in height using the walls of the nest box.
	The cup form is divided into:	
	4. Half cup	The nest material has a depression-like structure, but has not been built up extensively in height — no more than half the nest box height.
	5. Full cup	The sides of the nest extend more than half way up the height of the walls of the nest box.
	6. Hutted nest	The nest is provided with a roof of nesting material.

Table 3 Experiment II: experimental set-up and enrichment routines over the four periods.

Experiment II	Enrichment	Period 1	Period 2	Period 3	Period 4
Set-up	Bedding	200 g on cage floor	200 g on cage floor	200 g on cage floor	200 g on cage floor + 100 g on the nest box
	Wood wool	None	None	100 g in cage	100 g in cage
	Gnawing stick	2	1	1	1
	Straw	None	On the cage lid above the nest box roof	On the cage lid above the nest box roof	In the cage and on the cage lid above the nest box roof

study as we considered it possible that straw would provide a better structure to the nest than paper and wood wool alone.

Table 1 shows the details of the experimental design. The cage was cleaned once every two weeks; the nest was removed after examination and the rats and nest box were transferred to a clean cage. Because of soiling and the reduced cage-cleaning regime (cages were normally changed twice per week) additional fresh bedding material and straw was added twice per week, and wood wool and gnawing sticks once per week (see Table 1). The wooden box was machine-washed every two months. Figure 1 shows a clean and a soiled box.

The aim of Experiment I was to monitor whether the male rats would build nests, and if so, how they were shaped, if nest-building ability improved with time and if strain-specific differences existed. Every seventh day the box was removed and the nest weighed and scored using the score sheet described in Table 2. Photographs of special features were taken for documentation purposes.

Figure 1

A clean nest box and a soiled nest box in Macrolon™ IV cages (for the photograph, the nest boxes have been placed in order to obtain a good view of both entrances).

Experiment II

Four enrichment routines were run over three consecutive 14-day periods to test whether nest-building activity of the rats varied with different enrichment conditions and strains; see Table 3 for details. Every seventh day a descriptive evaluation was performed. The use of the gnawing sticks was assessed using the number of bite marks on the sticks. In periods 2 and 3 (Table 3), straw was placed on top of the cage lid. Placing wood wool on the cage lid is a normal enrichment procedure in our laboratory; however, it had been observed that BDIX rats could not, or would not, pull the wood wool into the cage (unpublished data). In this experiment we wanted to document if this above mentioned behaviour was seen with straw also. In period 4, bedding was added to the nest box roof because observations from our laboratory (unpublished data) showed that this might cause the animals to avoid the nest box roof.

Animals and housing

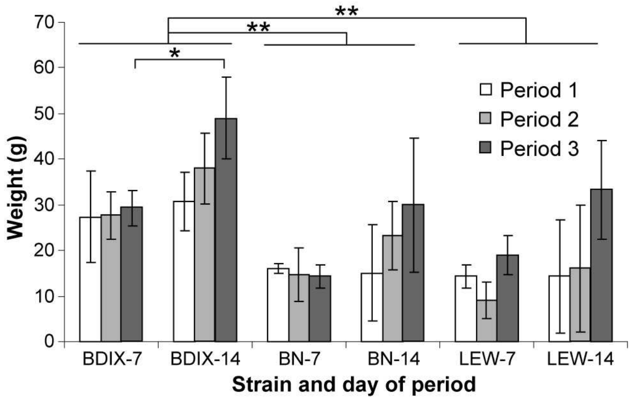
When the animals arrived at the laboratory they had a certified health report according to the Federation of European Laboratory Animal Science Associations (FELASA) recommendations, indicating that no pathogens

Table 4 Experiment I: The number of nests scored at each stage of construction (three replicate cages per strain) over the three periods. The digits in the left column refer to building complexity (see Table 2).

Strain	BN						BDIX						LEW					
Period divided by cage cleaning	1		2		3		1		2		3		1		2		3	
Day	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14	7	14
6+5+4+3+1																		
5+4+3+1						1		1				3						
4+3+1		1	1	3		2	1	1	1	3	1					1		
3+1	1	1	1		3		2	1	2		2		2	1	1	1	3	3
2+1	2	1	1										1	2	2	1		
1																		

Advancing stages ↑

Figure 2



The accumulation of nesting material inside the nest box during three consecutive 14-day periods.

were present (for detailed specification see Kraft *et al* 1994). The animals were kept under standard laboratory conditions: room temperature 20–24°C, relative humidity 50–60% and a 12:12 h light:dark cycle (lights on 0600h–1800h). All animals had free access to tap water supplied in water bottles, which was changed once per week. The diet (Altromin 1314: Chr Petersen Inc, Denmark) was available *ad libitum*.

Statistics

The accumulated weight of the nest material was analysed using ANOVA. The result was considered statistically significant when $P < 0.05$. The complexity was analysed using ordinal logistic regression, modelling the complexity (in the order shown in Table 4) as a function of time (14 versus 7 days), period (1–3), and strain of rat.

Results

Experiment I

Throughout the experiment, all three strains of rat were observed using the roof of the nest box (results not presented). All pairs of rats exhibited nest-building activity, and at any time all cages contained a nest that could be identified at some stage of construction. Table 2 describes the different stages of nest construction that were scored, and Table 4 presents the number of nests found at each stage of construction for the three strains of rat, after days 7 and 14, over the three periods. All aspen bedding material was removed from the nest box by the rats before they started to construct the nest using the nest materials.

Before the rats started to build the nest, the nest material was scattered on the floor of the cage, without any clear structure. Collection of the nest material by the rats led to the formation of a mat inside the nest box that could be up to several centimetres thick. The mat could also be built up further to form a cup-shaped nest. In more advanced stages, the nest materials could reach as high as halfway up the inside of the nest box. The structure of the nests consisted of fibres of both straw and wood wool. These fibres were long and often had an interwoven appearance. The subjective impression was that straw was a more favourable building material than wood wool, as straw clearly formed a larger part of the nest structure itself. The nest material was not only found in the nests, but also found in large amounts scattered within the bedding material. This nest material was in very small pieces and could indicate a dual use of the material: for gnawing and nesting.

The results of Experiment I are presented in Table 4. After 7 days in period 1, the BN rats had two nests that were without structure (stage 2) and one nest was a mat (stage 3); after 7 days in period 2, one nest was without structure, one was a mat, and one had become a half cup (stage 4); and after 7 days in period 3, all three nests were mats. After 14 days in period 1, one nest was still without structure, one was a mat, and one had become a half cup; after 14 days in period 2, all three nests were a half cup; after 14 days in period 3, two nests were built as a half cup and one nest had become a full cup. There appeared to be an increase in the complexity of the nests after the first 7 days of each period and again after 14 days of each period. Overall, the BN rats appeared to become more skilled and quicker in building the nests over the three periods.

After 7 days in all three periods, the BDIX rats had two nests that were mats and one nest that was a half cup. After 14 days in period 1, one nest was shaped as a mat, one as a half cup, and one as a full cup. After 14 days in period 2, all three nests were shaped as a half cup, and after 14 days in period 3, all nests were full cups. The BDIX rats did not appear to become more skilled in building the initial stages

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Table 5 Experiment II: Set-up and results of enrichment routines.

Experiment II	Enrichment	Period 1	Period 2	Period 3	Period 4
Set-up	Bedding	200 g on cage floor	200 g on cage floor	200 g on cage floor	200 g on cage floor + 100 g on the nest box
	Wood wool	None	None	100 g in cage	100 g in cage
	Gnawing stick	2	1	1	1
	Straw	None	On the cage lid above the nest box roof	On the cage lid above the nest box roof	In the cage and on the cage lid above the nest box roof
Results	**BN**	Some use of gnawing stick (Figure 3)	All 3 pairs had used the straw for nests Nest in all cages	None of the 3 pairs had used the straw on the lid, but all had used the wood wool Nest in all cages	The bedding had not been touched, meaning none of the animals had been on the roof in all 3 cages; straw had not been moved from the cage lid (see Figure 4 a–c) Nest in all cages
	BDIX	No use of gnawing stick (Figure 3)	All 3 pairs had not moved the straw No nest seen	None had moved the straw on the lid, but all had used the wood wool Nest in all cages	The bedding had gone from the top of the nest box, but the straw had not been moved from the cage lid (see Figure 5) Nest in all cages
	LEW	Some use of gnawing stick (Figure 3)	All 3 pairs had moved the straw into the cage Nest in all cages	All 3 pairs had moved and used the straw and the wood wool Nest in all cages	Bedding had gone from the top of the nest boxes and straw had gone from the cage lid in all 3 cages (see Figure 5) Nest in all cages

of the nest construction in the first 7 days, but progressively more advanced nests were recorded after 14 days over the three periods. The BDIX rats also used significantly more nest material by weight compared with the BN and LEW rats during the first 7 days and for the entire 14 days ($P < 0.01$) (see Figure 2).

After 7 days in period 1, the LEW rats had two nests that were mats and one that was still without structure; after 7 days in period 2, two nests were without structure and one was a mat; after 7 days in period 3, all nests were mats. After 14 days in period 1, one nest was a mat and two nests were without structure; after 14 days in period 2, one nest was without structure, one was a mat and one had been turned into a half cup; and after 14 days in period 3 all nests were still mats. The LEW rats improved their building ability in the initial stages but not in the more advanced stages (Table 4).

All three strains had a tendency to construct more complex nests after 14 days compared with after 7 days, and with increasing complexity over the three periods. Within each strain, these tendencies were significant for the difference between 14 and 7 days in BN and BDIX rats ($P < 0.001$ and $P = 0.013$ respectively). In a joint analysis of all three strains, the effects of both time (14 days versus 7 days) and period (1–3) on complexity were significant ($P < 0.001$ and $P = 0.001$ respectively). Furthermore, differences between the three strains of rat were significant ($P = 0.002$).

Figure 3

Experiment II: The use of gnawing sticks in period 1. One stick per cage per week was provided; the first and second weeks are marked with I and II respectively.

Experiment II

When nest material (wood wool or straw) was not provided (see Table 5, period 1), BN and LEW rats made some use of the gnawing stick. In contrast, no signs of gnawing were seen on the sticks of the BDIX rats (see Figure 3).

Differences were also found in the behaviour of the three rat strains depending on where the nest and bedding materials were placed in the cage. The LEW rats continued to use the roof of the nest box and the nest material, regardless of where the nest and bedding material was placed in the cage (Table 5, periods 2–4). The BDIX rats did not use the straw

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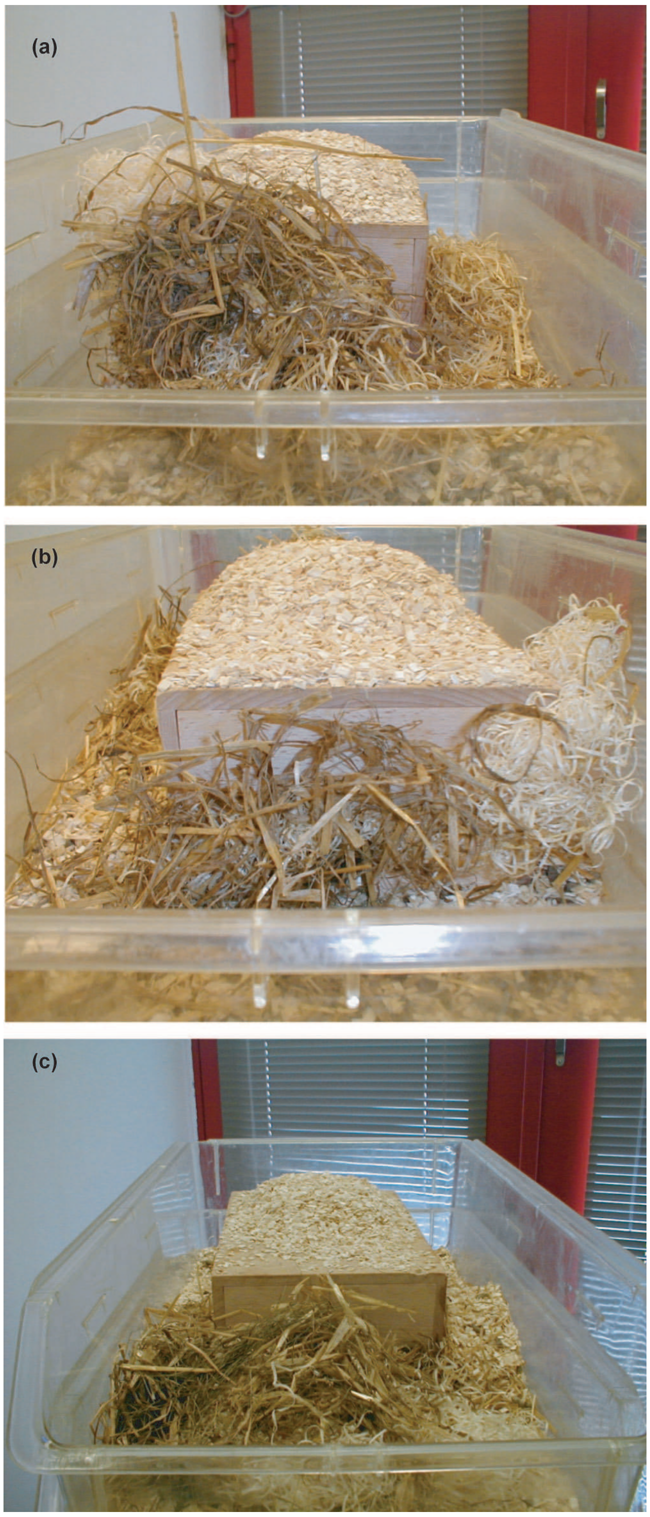
Figure 4



Experiment II, period 4. BN rats used nest material to hide the entrance openings. **(a–c)** Cages of all three pairs of BN rats are shown.

when it was placed upon the lid of the cage, even if no alternative nest material was available (Table 5, periods 2–4). The BN rats used the straw placed on the lid of the cage for nest-building when no alternative nest material was provided inside the cage; however, when nest material was available inside the cage the rats did not use the straw on the lid of the cage (Table 5, periods 2–4). Furthermore, all the BN rats avoided the nest box roof if bedding was placed on top of the box, but this was not the case for the two other strains (Table 5, period 4). In addition to nest-building, the BN rats also used the nest materials to cover the two entrances/exits to the box (see Figure 4 a–c). This material had not been modified, as the wood wool was still in a curly structure and the straw was still long and straight.

Discussion

When the three inbred strains of laboratory rat were provided with a large nest box and with straw and wood wool as nest material, purposeful nest-building activity was observed. During the study, each pair of rats produced six nests, with the exception of the BDIX rats which produced five nests; nest-building began again each time the cage had been cleaned. In this study the rats exclusively used the nest box as their nest site. The lack of nest-building activity reported in previous studies (Lawlor 2002; Van Loo & Baumans 2004) may have been because nest boxes were not provided.

The nests of all three strains appeared similar and the same stages in nest construction could be identified, probably because of the innate fixed action patterns of nest behaviour. Eibl-Eibesfeldt (1961) outlined the three main elements of nesting behaviour: digging of the burrow; foraging for nest material; and building of the nest. The first phase, digging of the burrow, involves the scratching, kicking, turning, and pushing of dirt. In this study, the removal of the bedding from the next box by all strains before a nest was constructed could be regarded as a 'vacuum activity', ie a response to being unable to dig a real burrow, or it could be an attempt to dig a 'depression' for the placing of nest material, as reported by Calhoun (1962). The building of the nest phase involves grasping and depositing, splitting straws along their length with the teeth, pushing material with the legs, pushing building material with the snout, scratching at the material to form a bowl, and turning and pushing to build up a ring-shaped mound (Eibl-Eibesfeldt 1961). The rats in this study had a time restriction of 14 days for nest-building; after 14 days, the nest was removed and they had to start again. Although we did not see the hutted nest constructed in this study, our rats were surprisingly adept. In the first experiment, straw and wood wool were provided inside the cage. Straw appeared to be the major component used to construct the nests and this may be because straw is easier to manipulate for nesting than wood wool. In a study by Blom *et al* (1996) it was concluded that rats choose materials on the basis of their manipulative properties, and the splitting of the material longitudinally, which is an innate behaviour (Eibl-Eibesfeldt 1961), is probably easier with a straight straw than with curly wood wool. Straw might also provide better support for the nest, or straw may be preferred as it is the material of choice for nest-building in the rats' natural environment (Calhoun 1962).

The foraging for the nest material phase involves grasping, pulling free, biting loose, carrying and depositing of nest

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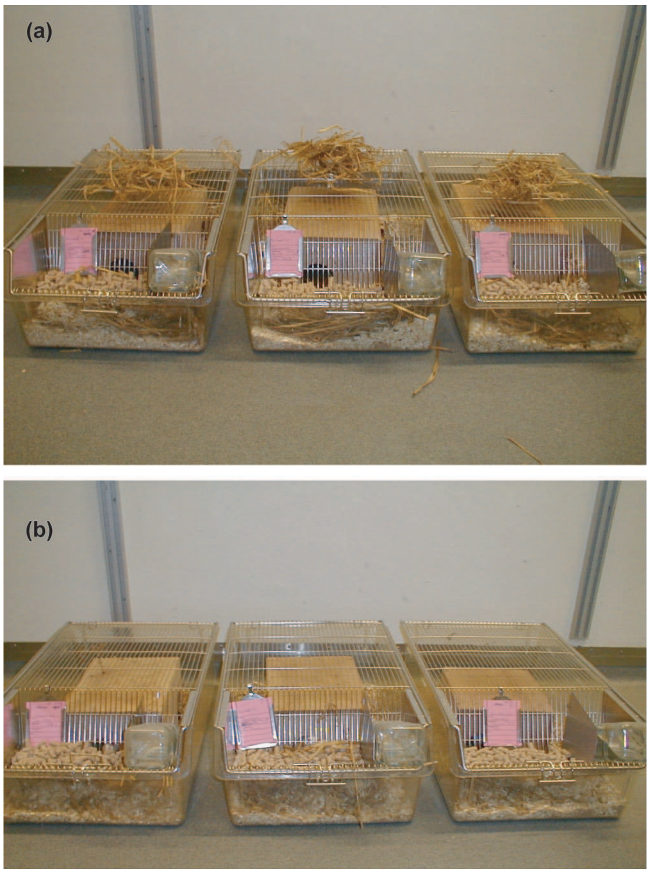
material (Eibl-Eibesfeldt 1961). In this phase, major differences between the strains were found during Experiment II. None of the six BDIX rats retrieved the straw from the lid of the cage (see Figure 5), even when no alternative nest material was provided. The BN rats did remove the straw from the cage lid when no alternative was given; however, if an alternative source of nesting material, such as wood wool, was provided inside the cage the BN rats would use this rather than collect the straw from the cage lid. Furthermore, if the nest box roof had bedding material on it, all six BN individuals stopped using the roof. The LEW rats used all the material available to them irrespective of its location. The behavioural differences between the three strains need further study in order to establish possible correlations with differences in anxiety level, motivational stage, release mechanisms, learning ability, or even physical abilities or disabilities (eg the straw might not be visually recognised or smelt by the BDIX rat). The lack of use of the gnawing stick by the BDIX strain is also a remarkable finding, especially in the absence of other material, eg wood wool and straw. Why the gnawing stick is not used by the BDIX rats, despite the absence of nest material, is unknown, and requires more thorough investigation.

This study showed that there were no additional enrichment benefits from placing the straw on the cage lid instead of inside the cage for the BDIX strain of rats. The complexity of the nest constructed by BN and BDIX rats increased significantly with time, with a similar trend seen in LEW; however, it is important to note that this improvement occurred over 14 days. A study with the SD strain of rats (Jegstrup & Ritskes-Hoitinga 2004) showed that they worked constantly on the same nest for three weeks, limited only by cleaning and the disposal of the built nests. It is important to determine the length of time required for the nests to be constructed, before a timescale of cleaning can be proposed. It is unknown if the motivation for nest-building would be maintained over time, if the cleaning procedure continually removed the nests; however, outbred SD rats maintained this motivation for more than 25 weeks, despite removal of the built nests, therefore it appears to be a stable behaviour (Jegstrup & Ritskes-Hoitinga 2004).

The BDIX strain of rats used significantly more nest material than the two other strains, which might result from a higher energy demand to maintain homeostasis under normal laboratory conditions compared with BN and LEW rats, and this needs further investigation. Kinder (1927) has shown that there is a close relationship between temperature regulation and the amount of nest material used; not only does the amount of material increase with the lowering of environmental temperature, but also it increases if the individuals have been pre-adapted to higher temperatures. In mice (Gordon 2004) it was found that different bedding material influenced core temperature and metabolic rate.

A remarkable trait observed in the BN strain was the use of nest material to completely cover the entrances of the nest box. In nature, the rat is prey for predators with excellent sight, such as birds and cats. Therefore, rats have evolved a survival strategy that includes nocturnal behaviour, hiding of their burrows, and covering of the entrances to their burrows. The similarity between the behaviour of the wild rat and the BN rat in this study is striking, and may indicate that the genetic relationship between the BN strain of laboratory rat and the wild rat is closer than that for the other two strains.

Figure 5



Experiment II, period 4, **(a)** BDIX and **(b)** LEW. BDIX rats do not remove the nest material from the cage lid (all three replicate cages shown in the photographs).

Conclusion

Our study shows that male laboratory rats from three inbred strains have retained their ability to exhibit nest-building behaviour given the correct stimuli, and that nest-building behaviour is not limited to females. However, it is necessary to provide nest material of suitable structure, in an appropriate way, to motivate the rats in this behaviour. When provided with a large nest box and nest material of straw and wood wool, male rats of three inbred strains make nests very similar to those seen in nature. This form of enrichment has also been shown to reduce aggression (Jegstrup & Ritskes-Hoitinga 2004), as it reduces boredom and adds a self-made construction to the cage in which the rats can hide and rest. However, strain-specific differences need to be taken into consideration when enrichment practices are implemented — for example, nest material placed on the cage lid will not be used by BDIX rats. Therefore, husbandry procedures should be adapted effectively to meet strain-specific needs, and should benefit the welfare of all individual animals involved. For this reason we recommend that all cages for rats be provided with large nest boxes and nest material that at least includes straw.

Acknowledgements

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