Institut für Genetik, Ernährung und Haltung von Haustieren,
Abteilung Tierhaltung und Tierschutz,
der Vetsuisse - Fakultät der Universität Bern
(Prof. Dr. A. Steiger)

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Behaviour of golden hamsters (*Mesocricetus auratus*) kept in four different cage sizes

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Katerina Fischer
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1 Zusammenfassung

Verglichen mit der geschätzten Territoriumsgrösse wildlebender Goldhamster (*Mesocricetus auratus*) werden domestizierte Goldhamster häufig in sehr kleinen Käfigen gehalten. Werden sie als Haustiere gehalten, sind die Käfige zwar meistens etwas grösser als Laborkäfige (Makrolon Typ 4 = 1'800 cm^2), aber dennoch relativ klein. Im Rahmen einer Expedition der Universität Halle (Deutschland) und Aleppo (Syrien) haben Gattermann et al. (2001) das natürliche Habitat von Goldhamstern untersucht. Bewohnte Hamsterbauten waren mindestens 118 m voneinander entfernt. In Anbetracht dieser Distanz erscheint die Käfiggrösse als wichtiger Faktor für das Wohlbefinden von Goldhamstern.

Ziel der vorliegenden Arbeiten waren Untersuchungen des Verhaltens und morphologischer sowie physiologischer Parameter bei der Haltung von Goldhamstern auf verschieden grossen Grundflächen. Weiterhin wurde der Einfluss verschiedener Stressfaktoren auf die Goldhamster untersucht.

Das erste Publikationsmanuskript *„Behaviour of golden hamsters (Mesocricetus auratus) kept in four different cage sizes*" (Kapitel 2) befasst sich mit dem Verhalten und mit weiteren Parametern bei Goldhamstern auf verschieden grossen Grundflächen. Mit Hilfe von Videoaufnahmen wurde das Verhalten von 60 weiblichen Goldhamstern, die in drei Durchgängen à 15 Hamster während 13 Wochen in Käfigen mit vier verschiedenen Grundflächen (1'800 cm^2, 2'500 cm^2, 5'000 cm^2, und 10'000 cm^2) einzeln gehalten wurden, verglichen und auf ihre Tiergerechtheit geprüft. Alle Käfige waren mit einem Holzhäuschen, einem Laufrad, einem Sandbad, Futterschale, Trinkflasche, Ästen, Heu, Papiertüchern und einer Kartonrolle

eingerichtet. Die Laufradaktivität wurde rund um die Uhr registriert. Eingestreut wurden die Käfige mit Hobelspänen, die 15 cm hoch eingefüllt wurden. Insgesamt wurden drei Videoaufnahmen (Wochen 3, 6, 10) gemacht und das Verhalten der Hamster in den verschieden grossen Käfigen wurde anschliessend ausgewertet. In Woche 11 und 12 wurden die Hamster an zwei aufeinander folgenden Tagen in der Lichtphase (Schlafphase) gestresst (Wecken, „Handling“, Herumjagen, Austauschen der Einstreu, Lärm, etc.). Zu 4 verschiedenen Zeitpunkten (Wochen 0, 2, 8, 13) wurden die Tiere gewogen. Am Schluss wurden die Hamster euthanasiert, es wurde Blut gewonnen und die Cortisol-, Corticosteron- und ACTH-Konzentrationen wurden gemessen.

In den vier Käfiggrössen konnten keine signifikanten Unterschiede in der Laufradaktivität festgestellt werden. An den zwei Tagen nach den Stress-Prozeduren liefen die Hamster signifikant länger im Laufrad als an den zwei Tagen vor dem Stress und während dem Stress. Stereotypes Gitternagen, häufig als ein Indikator für ungenügende Haltungsbedingungen verwendet, wurde in allen vier Käfiggrössen beobachtet. Mit zunehmender Käfiggrösse haben die Frequenz und die Dauer von stereotypem Gitternagen signifikant abgenommen. Die erhöhte Plattform auf dem Holzhäuschen wurde vermehrt von Hamstern aus den kleinen Käfigen genutzt. Dies könnte durch ein grösseres Bedürfnis nach zusätzlichem Platz erklärt werden. Die Gewichtszunahme bis zum Projektende war signifikant höher je kleiner der Käfig, beruhte aber nicht auf einem höheren Fettansatz der Tiere. Da sich die Hamster jedoch noch im Wachstum befanden, besteht die Möglichkeit, dass die überschüssige Energie zu einem späteren Zeitpunkt zu verfetteten Tieren führen könnte.

Aus den Resultaten schliessen wir, dass ein Käfig von 10'000 cm^2 oder grösser für Goldhamster zu empfehlen ist. Die Käfige können zudem mit mehr Strukturen angereichert werden, so dass die Hamster mehr Beschäftigungsmöglichkeiten haben. Eine tiefere Einstreu ist ebenfalls zu empfehlen, wie aus der parallel durchgeführten Dissertationsarbeit von A. Hauzenberger (2005) entnommen werden kann. Zusätzlich empfehlen wir, dem Goldhamster ein Sandbad zur Verfügung zu stellen, da es von allen Hamstern, unabhängig von der Käfiggrösse, sehr häufig genutzt wurde und für ihr Wohlbefinden von Bedeutung zu sein scheint.

Das zweite Publikationsmanuskript *„Can we tell hamsters are stressed by measuring their cortisol levels?“* (Kapitel 3) befasst sich mit den Schwierigkeiten, einen allfälligen Stresszustand mittels physiologischer Parameter zu erfassen. Bei den weiblichen Tieren im Versuch wurden signifikante Unterschiede in den Hormonkonzentrationen Cortisol, Corticosteron und ACTH zwischen den drei Versuchsdurchgängen gefunden. Die Käfiggrösse hatte keinen Effekt auf die Hormonwerte. Die Hormonwerte für Goldhamster sind in der Literatur uneinheitlich und zeigen grosse Varianzen auf. Die Hormonwerte im Blut sind sehr empfindlich gegenüber äusseren Einflüssen. Physiologische Stressmessungen müssen deshalb mit Vorsicht interpretiert werden und sollten immer im Zusammenhang mit anderen Parametern, z. B. dem Verhalten, beurteilt werden.

2 Behaviour of golden hamsters (*Mesocricetus auratus*) kept in four different cage sizes

This manuscript will be submitted to *Animal Welfare*

Behaviour of golden hamsters (*Mesocricetus auratus*) kept in four different cage sizes

Katerina Fischer, Sabine G. Gebhardt-Henrich, Andreas Steiger

Vetsuisse Faculty Bern, Institute of Animal Genetics, Nutrition and Housing, Division of Animal Housing and Welfare, P.O. Box, CH-3001, Bern, Switzerland

Correspondent author:
Katerina Fischer, Vetsuisse Faculty Bern, Institute of Animal Genetics, Nutrition and Housing, Division of Animal Housing and Welfare, Bremgartenstrasse 109a, P.O. Box, CH-3001 Bern, Switzerland
Phone: +41-31-631-23-66
Fax: +41-31-631-26-40
e-mail: katerina.fischer@itz.unibe.ch

Abstract

Cages for laboratory hamsters as well as for pet hamsters are usually quite small. Using video recordings, the behaviour of sixty female golden hamsters (*Mesocricetus auratus*) housed in four different cage sizes was compared in order to draw conclusions about their welfare. The cage sizes were 1,800 cm^2, 2,500 cm^2, 5,000 cm^2, and 10,000 cm^2. Enrichment items and litter depth (15 cm) were standardised and all cages were equipped with a running wheel. There were no significant differences in running wheel activity. In all cage sizes stereotypic wire gnawing was observed, but the hamsters in the small cages did it significantly longer and more frequently. In small cages more hamsters used the additional platform of their wooden houses than in big cages, which could suggest that they need more space. Therefore, we recommend cages with a minimal ground floor area of 10,000 cm^2 for golden hamsters.

Keywords: animal welfare, golden hamster, cage size, stereotypy, wire gnawing, running wheel

Introduction

Hamsters are common laboratory animals in biomedical research as well as popular pet animals. Nevertheless, little work has been done with the specific intent of improving their housing conditions, especially for pet animals. Exceptions are the work by Kuhnen (1999a), Bantin and Sanders (1989) on cage size, Mrosovsky et al. (1998) on running wheel choice by Syrian hamsters, and Reebs and Maillet (2003) on environmental enrichment. Reebs and Maillet (2003) showed that there were fewer daily revolutions, shorter wheel running activity phases, and delayed running activity onsets in hamsters housed in multiple-cage systems enriched with a running wheel and commercial wooden toys compared with hamsters housed in single cages with a running wheel, without other enrichments. Mrosovsky et al. (1998) found that golden hamsters ran more in wheels with the floor covered by a plastic mesh than in wheels with the usual rods. This preference was evident both in tests with a single wheel and in tests when the animals were offered a choice between two wheels. In the case of Kuhnen (1999a), golden hamsters were individually housed in four different cage sizes from 200 cm² to 1,815 cm². Increasing cage size increased the mean febrile response, while the mean baseline rectal temperature decreased. The results indicate that housing in small cages induces chronic stress, which affects thermoregulation. The cages are common for laboratory rodents. Pet golden hamsters are usually kept in cages at least as big as a Macrolon 4 type laboratory cage, which is 1,800 cm². In Switzerland this is the statutory minimum size according to the actual ordinance for the protection of animals. The cage size is of great importance for the welfare of the animals, as shown in the studies mentioned above. The pets spend their whole life in their cages and should have the possibility to perform all their behavioural needs. Stress levels should be kept as low as possible.

In a joint expedition by the universities of Halle (Germany) and Aleppo (Syria), Gattermann et al. (2001) explored natural hamster habitats. The closest distance between occupied hamster burrows was 118 m. A mean tunnel length of 199.5 ± 92.6 cm and a mean burrow depth of 64.8 ± 17.6 cm were observed (Gattermann et al., 2001). This shows that the natural territory of a hamster is much bigger than any cage. Laboratory hamsters compared with wild caught hamsters did not show any differences in behaviour (Gattermann, 2000). Despite domestication for decades, they are capable to survive in a semi natural environment as demonstrated by Gattermann (2000). Therefore, domesticated hamsters might need more space than we commonly offer them.

The aim of this study was to show differences in the behaviour of golden hamsters housed in different sized cages and to draw conclusions about their welfare. Furthermore the response of the running wheel activity to different stressors was analysed. The study focused especially on pet hamsters, therefore cage sizes used were much bigger than common laboratory cages. Areas of cages used in this study were 1,800 cm², 2,500 cm², 5,000 cm² and 10,000 cm². The area of the smallest cage was chosen because it was the statutory minimum in Switzerland. An area of 2,500 cm² is a commonly used size. The Swiss Animal Protection (SAP) demands a minimum area of 5,000 cm² and they recommend a cage of more than 10,000 cm² (Lerch-Leemann, 2002).

Methods

Animals and housing conditions

The sixty female golden hamsters used for this project were progeny of the strain Crl: LVG (SYR) from Charles River, Germany. During one year the sixty hamsters were bred in three series of 20 hamsters each. A photoperiod of 12h light, 12h dark, dawn at 1300h was maintained. The light decreased from 280 Lux within half an hour to maximal 5 Lux. Room temperature was 21±2°C, relative humidity was not regulated, but was between 25% and 59%. Hamsters were bred in cages with a wire top and plastic bottom (length x width x height: 95 cm x 57 cm x 45 cm) without a running wheel. At the age of 24 to 30 days hamsters were placed singly into four different sized cages (App. I to III). Size 1 was: 32 cm x 57 cm x 45 cm (length x width x height), (approx. 1,800 cm^2); size 2: 44 cm x 57 cm x 45 cm, (approx. 2,500 cm^2), size 3: 95 cm x 57 cm x 45 cm, (approx. 5,000cm^2), and size 4: 105 cm x 95 cm x 45 cm (approx. 10,000 cm^2). All cages were furnished with a wooden nest box (20 cm x 14 cm x 14 cm), litter (depth: 15 cm), hay, paper towel, cardboard tubes, twigs, a sand bath (diameter: 16 cm) and a running wheel (diameter: 30 cm, width: 10 cm). Commercial pet hamster food (Witte Molen®, NL-Meeuwen) and water were offered ad lib. This diet was amended by dry cat food and vitamin and mineral supplements (Marienfelde Vitakalk). In addition, fresh fruits and vegetables were added every day. Litter was never changed completely. Only the dirty parts of the litter were changed when necessary. Due to space limitations, the project was made in 3 series. Five cages of each size were used simultaneously. In each of the three series, 20 hamsters were distributed singly in the 20 cages. If possible, hamsters of one litter were placed randomly, but according to their body mass, in cages of 4 different sizes. The experiment was approved by the Cantonal Office of Agriculture and Nature.

Procedure and Measurements

Procedure

In week 0, at weaning, hamsters were approximately 4 weeks of age. During the experiment they were weighed repeatedly (Fig. 1). The body length (snout to tip of tail) was measured before termination on the anaesthetized animal, which was stretched out on a ruler. The body condition of the hamsters was calculated as body weight in proportion to body length (body weight in week 13/length^3), which is a degree of fat tissue. Hamsters were video-taped, between 15:30 and 18:30 p.m., i.e. 2:30 hours after dawn (Fig. 1). In weeks 11 and 12 each hamster was stressed on two following days by shaking the cage, chasing, handling, habitation in a small cardboard box for 30 minutes and loud music for three to five minutes. Additionally on the first day of stress half of the litter was changed, while on the second day a confrontation with another hamster was performed. Thirteen weeks after weaning hamsters were decapitated after Isoflurane-anaesthesia. Blood was collected and sent to Alomed, Analytical Laboratory Dr. W. Müller, (Germany), for analyses of corticosterone, cortisol and ACTH. Organs (adrenals, heart, liver, kidney and spleen) were weighed and checked for differences in the 4 cage sizes. Special attention was given to the weights of adrenals as an indicator of stress. Gastric mucosa was controlled for ulcers. All brains were examined for hydrocephalus internus.

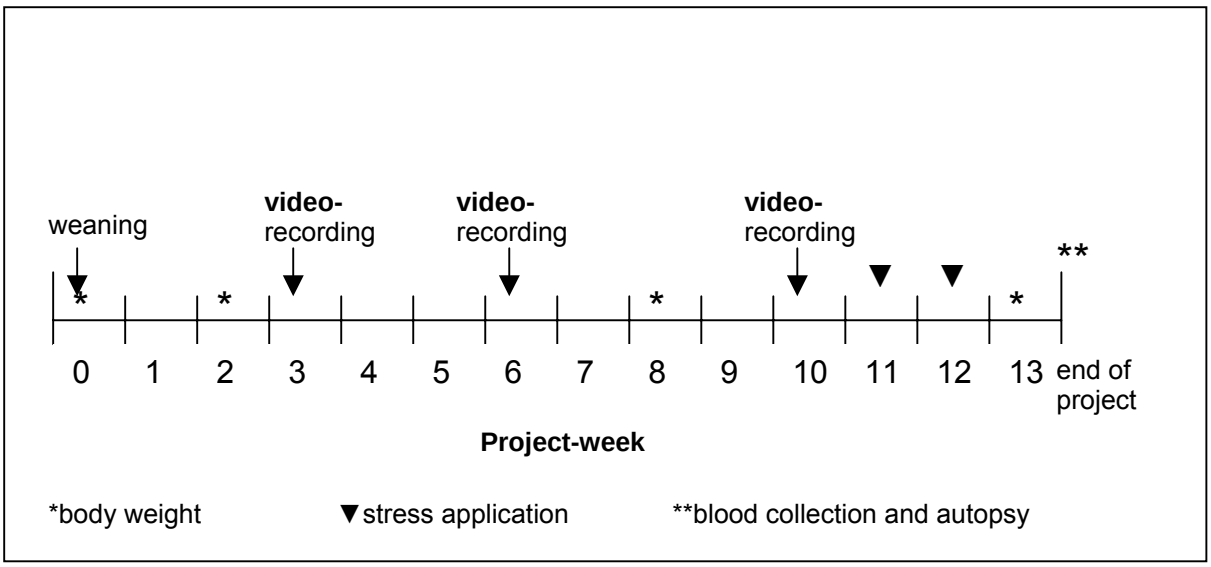

Fig. 1 Time table of the experiment. The stress treatment was conducted over 2 weeks.

Running-wheel activity

Revolutions of running wheels were constantly registered by the *Chronobiology Kit*™ (Stanford Software Systems). For the analysis of the running-wheel activity we used the median of the daily revolutions until week 10, before the hamsters were stressed. From time to time some hamsters blocked the running-wheel with litter and in some cases the running-wheel was not functioning or the transmission of data failed. These data were excluded from the analyses. Furthermore, all running-wheel data of one hamster from cage size 2 was excluded because the transmission of the running-wheel data failed consistently. For analyses of the effects of stress the mean number of revolutions of the 2 days during stress, as well as 2 days before and 2 days after stress, were analysed.

Behaviour

A total of thirty minutes of the taped behaviour was analysed using the Observer™ *Version 5.0* (Noldus). The thirty minutes of observation were split into six five-minute sequences during which the hamster stayed outside the house, i.e. the hamster was not sleeping and was visible. If possible, the sequences were spaced equally over the three recorded hours. If a hamster was not active or visible during the whole 3 recorded hours, so that observation sequences could not be spaced equally, a total of thirty minutes of the time the hamsters were visible on the tape was analysed. In some cases (4%, 7 hamsters, each with 1 observation) hamsters were active for less than 30 minutes, thus their observational data was based on the time they were active. One of the hamsters in cage size 2 was never active during the recorded time, so behavioural data for this animal are missing. The behavioural data were expressed as the percentage of total observed time for all three observations (total percent duration). For each observation separately a repeated measure analysis was made. Mean duration (sec.) and frequency (bouts) were analysed, when the percent duration suggested interesting results. Behaviours and locations were classified as described in App. IV, following Vonlanthen (2003).

Statistics

All statistical analyses were made with NCSS 2001, or SAS. Data and residuals were checked for normality and transformed if necessary and possible. Transformations and tests are described in the results. The series and the occurrence of hydrocephalus were included as factors in the analyses of behavioural data and are mentioned if they were significant. Correlations were calculated by using Spearman rank correlations coefficients.

Results

Behaviour

Hamsters devoted most of their observed activity to wheel-running. The remaining time was spent mainly on resting, rearing and grooming (Fig. 2). Correlations between the different behaviours (Tab. 1) were mainly positive, which indicates that the hamsters were generally active and showed various behaviours. However, the more time a hamster spent wheel-running, the less it spent with other activities. Likewise, the more time a hamster spent wheel-running, the less time he spent wire gnawing or climbing. In contrast, climbing was positively correlated to wire gnawing. The important results of the different behavioural activities are listed below. The occurrence of hydrocephalus as a main factor was never significant.

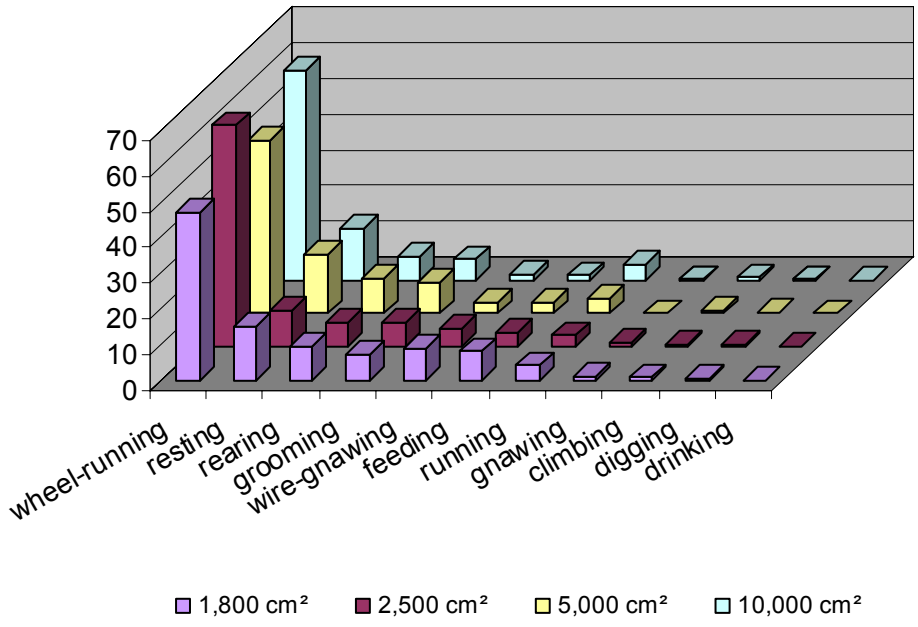

Fig. 2 Total percent duration of observed behaviours in the different cage sizes.

Tab.1 Correlation between the durations of various behaviours

+ or - correlation r_s>0.3, ++, or -- correlation r_s > 0.6, +++, or --- correlation r_s > 0.9, blank fields show no significant correlation.

Behaviour	wheel running	resting	rearing	grooming	wire gnawing	feeding	running	gnawing	climbing
wheel running		-	- -		- -	- -	- -	-	- -
resting	0.000038		+					+	
rearing	0.000000	0.002952			++	+	+	+	+
grooming									
wire gnawing	0.000000		0.000000			+++	+	+	++
feeding	0.000000		0.000001		0.000000		+	+	++
running	0.000000		0.000433		0.000132	0.000397		+	++
gnawing	0.000109	0.009750	0.011624		0.000588	0.001295	0.002035		+
climbing	0.000000		0.000001		0.000000	0.000000	0.000000	0.001828	

The important results of the different behavioural activities are listed below. The occurrence of hydrocephalus as a main factor was never significant.

Running-wheel

All hamsters used the running-wheel. The average distance was 8.3 km per day (8872 revolutions). The minimum per animal was on average 0.63 km per day; the maximum was 18.56 km per day. During the 10 weeks before the stress treatment, running wheel activity of hamsters was similar in all 4 cage sizes ($F = 0.88$, $N = 59$, $P = 0.4552$). The stress treatment affected running wheel activity significantly in all cage sizes. During the two days after stress treatment running-wheel activity was significantly higher than before and during stress treatment (Repeated Measures-ANOVA: $F = 5.31$, $N = 45$, $P = 0.0068$) (Tab. 2).

The total percent duration of wheel-running seen in the observations correlated with the median of the revolutions per day measured with the *Chronobiology Kit* ($r_s = 0.60$, $N = 58$, $P < 0.0001$).

Tab. 2 Post-hoc comparisons of running-wheel activity 2 days before (2BS), 2 days during (2DS) and 2 days after (2AS) stress treatment.

Comparison	T-Value	p-Value	Revolutions/day
2AS vs. 2BS and 2DS	T = 3.2059	p = 0.001823	
2AS vs. 2BS	T = 2.2127	p = 0.029267	+1188
2AS vs. 2DS	T = 3.3436	p = 0.001177	+2115.36
2BS vs. 2DS	T = 1.1617	p = 0.248200	+927.36

Wire gnawing

Compared with gnawing at various structures (cardboard tube, twigs, house, etc.) the hamsters generally gnawed at the wire for longer periods (Wilcoxon Signed- Rank Test: Z = 4.3439, P < 0.0001). While 13 (22%) of 59 hamsters showed both behaviours, 17 (29%) showed only wire gnawing and 3 (5%) gnawed exclusively on other material besides the wire. Although in the smallest cages the number of hamsters observed wire gnawing was twice as high as the number of hamsters which were never observed wire gnawing (App. V), there was no significant effect of cage size on the number of wire gnawing hamsters. For further analyses a minimum threshold for duration (1% of the total observed time) was defined (following Wiedenmayer, 1995, for digging in gerbils), to be able to define if the behaviour was stereotypic or not, which was the case for 22 out of 59 hamsters. Frequency of wire gnawing decreased with increasing cage size (square root transformation: F = 3.35, N = 22, P = 0.0454) (Fig. 3). Total percent duration of wire gnawing was significantly longer in smaller cages (Mixed model using REML, square root of 2 x arsin transformation: F = 14.00, N = 22, P = 0.0015) (Fig. 4). Comparing the smallest (1,800 cm^2) and the biggest cage size (10,000 cm^2) the difference was significant (Tukey's Studentized Range Test for percent duration: P < 0.05). Total percent

duration of wire gnawing was positively correlated with final body weight (r_s = 0.43, N = 22, P = 0.0434).

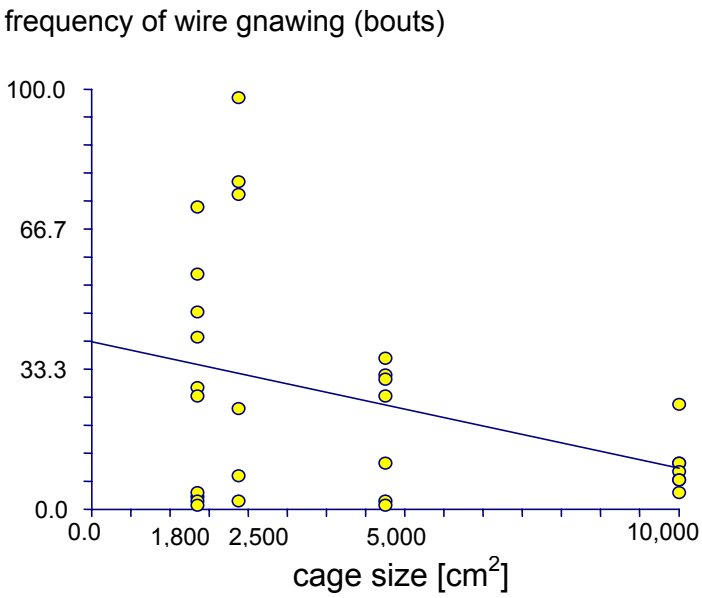

Fig. 3 Total frequency of wire gnawing in the different cage sizes. Raw data are shown, for the analyses the data were square root transformed.

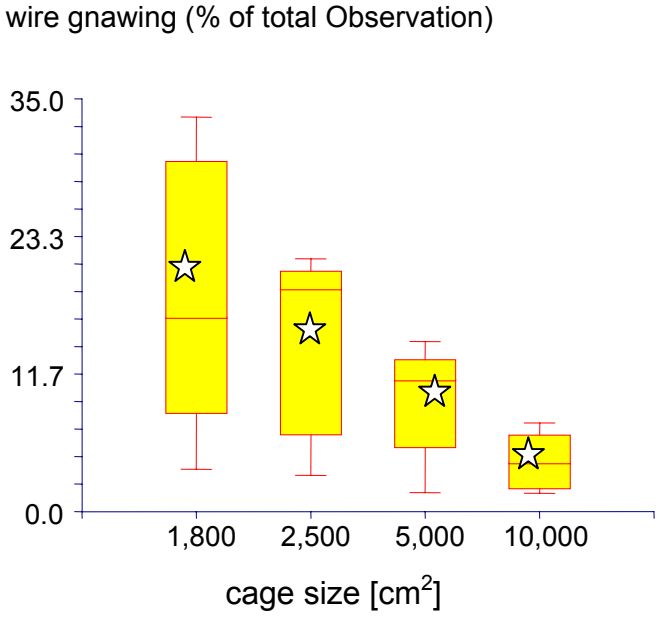

Fig. 4 Duration of wire gnawing (% of the total time of observation) in the 4 cage sizes. The box represents the middle 50% of the data, the horizontal line is the median. The vertical lines show 1.5 times the interquartile range; dots are values outside this range. The stars represent the mean value of total duration, which was 19.3 % for 1,800 cm^2, 14.5 % for 2,500 cm^2, 9.55 % for 5,000 cm^2 and 4.2 % for 10,000 cm^2. Raw data are shown, for the analyses the data were square root of 2*arsin transformed.

Grooming

All hamsters were observed grooming. Forty-six out of 60 hamsters showed grooming in the sand bath. A minimum of 0.02 % of total observed time and a maximum of 13.74 % of total observed time was spent on this behaviour. No significant differences were observed between grooming in the sand bath and grooming in other localities. Although the area of the sand bath was only 2 % of the area of the largest cage, hamsters in the biggest cages spent more than 12 % of grooming in the sand bath. In the smallest cages 19 % of grooming was performed in the sand bath while the area of the sand bath was 11 % of the cage area. But there were no significant differences between cage sizes (GLM-ANOVAs: $P > 0.1$).

There were no significant differences in the duration of running or resting among the four cage sizes.

Location

Hamsters stayed most of the observed time inside the running wheel (Fig. 5). The remaining time was spent in the open space, at the wire, in the food bowl or in the sand bath. Mostly positive correlations were found between the time that was spent in the locations shown in figure 5, showing that the hamsters used the whole cage and its structures (data not shown). The running wheel usage though was negatively correlated ($P < 0.007$) with the usage of these various locations.

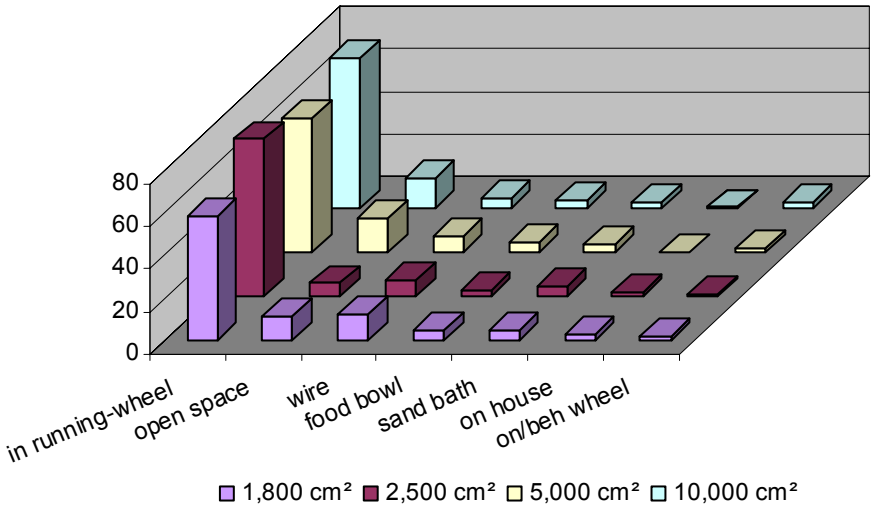

Fig. 5 Total percent duration of presence in most frequently visited locations in the different cage sizes. All other locations were visited rarely.

On house

More hamsters were observed on top of the house in small cages (χ^2_3 = 22.05, p < 0.0001, Tab. 3), but total percent duration as well as total frequency revealed no differences among cage sizes (P > 0.1)

Tab. 3 Number of hamsters observed on top of the house (X^2_3 = 22.05, p < 0.0001).

Cage size [cm^2]	observed on house	not observed on house	N
1,800	14	1	15
2,500	12	2	14
5,000	5	10	15
10,000	4	11	15
Total	**35**	**24**	**59**

Open space

Usage of open space was performed for longer time periods in big cages (F = 3.66, N = 59, P = 0.0187). There were no significant differences in the duration of usage of other localities.

Body weight

At week 0 body weights were similar in all four cage sizes (ANOVA, log-transformation: F = 2.65, N = 60, P = 0.143). The series had a significant influence on body weight (P < 0.05) with the exception of week 8 (Fig. 6). Hamsters from larger litters were significantly lighter throughout the experiment (Tab. 4). The effect of weaning age was significant in the first three body weight measurements, but not in week 13. Cage size never affected body weights significantly, but showed a tendency towards higher weights in big cages in week 13. Weight gain from weaning until week 13 was significantly smaller the bigger the cage (ANOVA: F = 4.03, N = 57, P = 0.013) (Fig. 7). Series, age of weaning and litter size had no effect on weight gain. The body condition (body weight in week 13 / length^3), was not significantly different between cage sizes (ANOVA: F = 1.93, N = 57, P = 0.1389). During autopsy, no difference in the amount of fat was noticed.

Tab. 4 ANOVAs of body weights at the 4 measured times with cage size and series as factors and litter size and age at weaning as covariates. N = 60, week 13: N = 57. No significant interactions occurred.

Project-week	Litter size		Weaning-age		Cage size	
0	F=6.59	P=0.0135	F=34.17	P<0.0001	F=0.63	P>0.5
2	F=11.07	P=0.0017	F=29.92	P<0.0001	F=0.76	P>0.5
8	F=9.96	P=0.0028	F=7.77	P=0.0077	F=0.84	P>0.4
13	F=5.30	P=0.0262	F=2.86	P=0.0979	F=2.47	P=0.0743

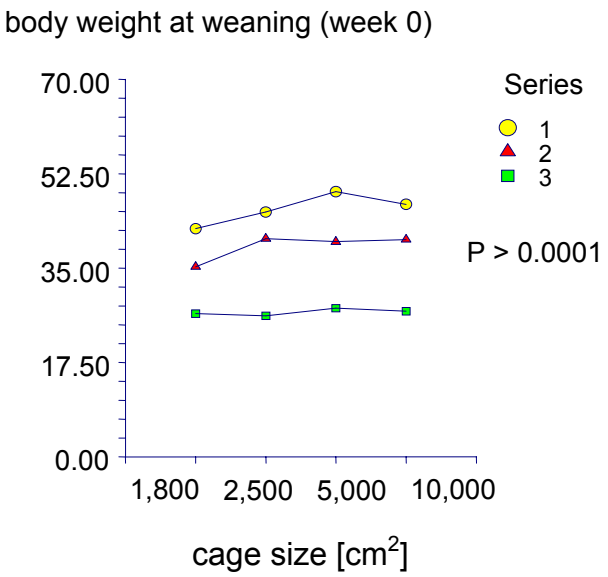

Fig. 6 Body weight at weaning in all cage sizes differed significantly among the three series: F = 35.14, N = 60, P < 0.0001 covariates were: litter size and weaning age. Raw data are shown, for the analyses the data were log-transformed.

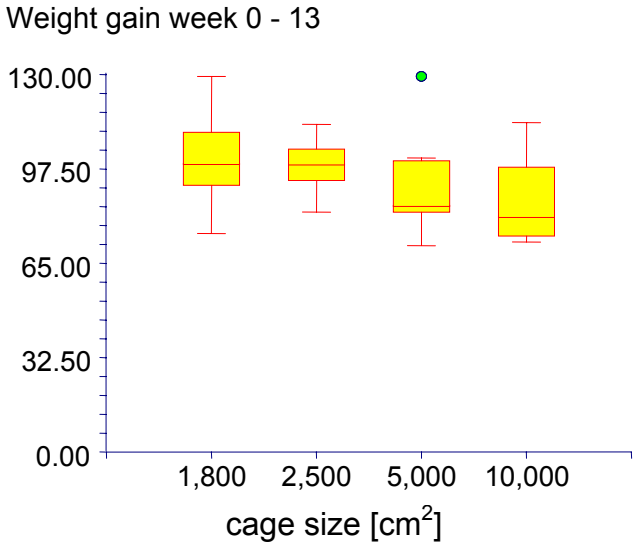

Fig. 7 ANOVA of weight gain from weaning to week 13 with cage size and series as factor variables and litter size and weaning age as covariates showed an effect of cage size, $F_{3, 43} = 4.03$, P = 0.013. The box represents the middle 50% of the data, the horizontal line is the median. The vertical lines show 1.5 times the interquartile range; dots are values outside this range.

Stress hormones and organs

Neither stress hormones nor the coefficient of cortisol/corticosterone differed between cage sizes ($P > 0.1$) (see also Gebhardt et al., in prep, Chapter 3).

No differences were found in organ weights including the weights of the adrenal glands.

Discussion

Hamsters spent most of the observed time wheel running. The running wheel activity was the same in all four cage sizes. This might indicate that running in the wheel is very important to them. A bigger cage obviously has no attraction that competes with wheel running. Gebhardt-Henrich et al. (2005) showed that the degree of wheel running was adapted to individual circumstances, since hamsters reduced or even stopped wheel running during breeding. Additionally, the running wheel had a beneficial effect on the well-being of the hamsters, since it significantly decreased stereotypic wire gnawing. Our results confirm their findings. The longer wheel running was performed, the less stereotypic wire gnawing was observed. Furthermore, hamsters which showed less wheel running seemed to compensate the need for movement with climbing. Nevertheless some scientists describe wheel running as a stereotypic behaviour (see review by Sherwin, 1998) and therefore it is not clear whether the provision of a running wheel should be mandatory for golden hamsters.

Stereotypies are common indicators for poor welfare (e.g. review by Mason 1991, Würbel 2001). Stereotypic behaviour is commonly defined by repetitive, unvarying behavioural patterns without obvious goal or function (Ödberg, 1987), when kept under barren housing conditions (Mason, 1991). Stereotypic wire gnawing is often observed in captive rodents (Würbel and Stauffacher 1996, 1997, 1998, Wiedenmayer 1997, Waiblinger 1999).

Wire gnawing of hamsters in the present study was considered to be stereotypic because it was repetitive, invariant and was performed at a particular spot on the wire top of the cage (similarly in mice, Würbel et. al. 1996). Additionally it was without function because wire gnawing was considered to develop from outside-

directed exploration, which, after a steady ineffectiveness, no longer contributed to exploration.

Wiedenmayer (1995) stated that 12 seconds was the critical minimum time for stereotypic digging in gerbils. Normal digging was never performed for the same amount of time. Similarly, we stated a total duration of wire gnawing of less than 1 % of total observation time as non-stereotypic, which is less than 54 seconds over an observation time of 90 minutes. Some hamsters were seen to bite into the wire shortly while passing the wire but then immediately walked on.

As shown in the results, hamsters clearly gnawed longer and more frequently on the wire than on other things. This was not expected, since it is not a natural behaviour of hamsters. Gnawing on the cardboard tube, on twigs or on the wooden house serves a purpose, since it is helpful for abrasion and cleaning of the teeth. Some hamsters fractionalised the cardboard tube and used it as nest material. Wire gnawing though seemed to be ineffective and senseless. It could not be prevented by providing natural material to chew on, so wire gnawing and gnawing at objects presumably had a different cause and / or function. Wire gnawing might be an attempt to escape from the cage (similarly in mice, Würbel et al. 1998a, 1998b).

The results from this study indicate that housing in big cages improved the welfare of the hamsters because it resulted in less stereotypic wire gnawing. The biggest cage with a size of 10,000 cm^2 was the one with the shortest wire gnawing duration and the lowest frequency (bouts). Duration and frequency of wire gnawing in 10,000 cm^2 was half of the duration and frequency in 5,000 cm^2 cages. If this is taken into account, the required minimum of the Swiss Animal Protection (SAP) of 5,000 cm^2 seems still to be too small. However, although hamsters in small cages showed more wire gnawing than hamsters housed in bigger cages, wire gnawing occurred in

all cages. This suggests that even a cage of more than 10,000 cm^2 is too small for female golden hamsters.

All cages were furnished with the same structures (enrichment). There was a lot of free space in the big cages, therefore the hamsters had the possibility to run more and faster. But the possibilities of preoccupation were the same in all cages. This could be another reason why wire gnawing was performed even in the big cages. It would be interesting to know whether stereotypic wire gnawing would persist in big cages with more enrichment (e.g. wooden toys, cork caves, climbing possibilities) and with a change of enrichments (Lambert et al., 2005, in mice). Ödberg (1987) found that an increase of cage size did not affect stereotyped jumping in voles, whereas enrichment with twigs reduced it. Spangenberg et. al. (2005) housed rats either singly in small cages (1,092 cm^2) with only one black plastic tube, or in groups in larger cages (3,938 cm^2) which were provided with more and various enrichment. Rats in the larger, more enriched cages displayed a more diverse behavioural repertoire which consisted of running, climbing and social behaviours. Compared with other studies, all cages in our study were enriched.

Litter depth should be considered as another possibility of enrichment in golden hamsters. Hauzenberger (2005) found that golden hamsters in a litter depth of 40 cm showed less stereotypic wire gnawing than hamsters housed in 10 cm deep litter, while in a litter depth of 80 cm no stereotypic wire gnawing was observed. All hamsters used the deep litter (either 40 cm or 80 cm) as a retreat possibility and built burrows. Running wheel activity was higher in 10 cm deep litter. This suggests that a combination of a big cage with deep litter could be a great improvement of the welfare of golden hamsters.

The positive correlation between wire gnawing and climbing can be explained by the preference of some hamsters to climb to a particular spot on the front or the

top wire. Some hamsters used to climb while pausing during wire gnawing. They usually climbed up and down the front side of the wire top but then returned to the same point and restarted wire gnawing. Outside directed exploratory climbing was considered as the behavioural source of stereotypic wire gnawing in laboratory mice (Würbel et al., 1996).

All hamsters used the sand bath for grooming regularly, but not exclusively. Most hamsters used to wallow in the sand. Thus a sand bath seems very important for the welfare of golden hamsters, whether housed in small or big cages. Certainly it can also be recommended for laboratory conditions as a reasonable enrichment, since the sand can be changed along with litter, if necessary.

The available free space was used in all cage sizes. Hamsters in big cages used the whole ground area and the observed duration which they spent in the open space was longer. They used to walk particularly along the walls, so that trails were formed. Staying near the walls (called thigmotaxis) is common in rodents and is used as an index of anxiety (Simon et al., 1994, in mice). Some hamsters built a big crop of litter in the middle of the cage and formed a trail through the middle of it, which led directly from the running wheel to the house.

Although there was more open space in the big cages and the distances between the locations were longer, so that hamsters could run more, running duration did not differ significantly among cage sizes. Possibly, hamsters in big cages ran faster, which led to a similar duration of running.

The occurrence of thigmotaxis is no argument against big cages. Due to the fact that there were only two possibilities for covering, the house and the space under and behind the running wheel, the hamsters could have been afraid of running in the open space. But big cages can be enriched more than small cages, so that more possibilities for covering can be given. Additionally more enrichment may lead to less

stereotypic behaviour and can improve animal welfare (e.g. Ödberg, 1987 in bank voles, Würbel et. al. 1998b in mice, Kuhnen 1999b in golden hamsters). The well being of caged animals is affected by many factors (Bantin and Sanders, 1989). Weiss et al. (in Bantin and Sanders 1982) showed that rats prefer to live in big narrow cages compared to big broad cages. Although our cages were much bigger, the shape of the cage could also be important for hamsters.

The additional space on top of the wooden house was used by almost all hamsters in the two smallest cages. On the contrary only a few hamsters in the bigger cages used the elevated platform. Although duration and frequency were not significantly different, it suggests that hamsters in the two smallest cage sizes were in need of additional space and did not use the roof of the house only because it was an elevated platform.

It was ensured that hamsters were distributed equally in the four cage sizes according to body weight at weaning. Due to different litter sizes in the three series, a significant influence of series on body weights was observed. Possible reasons for the higher weight gain in small cages could be lower energy consumption and/or more food intake. The analysis of the condition, as well as autopsy results showed that the higher weight gain was not due to more fat tissue. Faster running in big cages, which uses more energy, would explain higher energy consumption in big cages. Hamsters in smaller cages were thus able to spend more energy on growth. Hamsters were still growing throughout the project. With an advanced age excessive energy can not be used for growth anymore. Then the additional fat tissue could become a problem in small cages. The lack of a running wheel or another activity with high energy expenditure could impair the condition additionally. Therefore, cage sizes 1 and 2 seem to be too small.

Autopsies of the three hamsters that died during the project revealed that all of them had severe hydrocephalus internus, but there had been no symptoms to draw attention to these hamsters (Edwards et al., 2005). Hydrocephalus as a main factor never had a significant effect on any analysis. Nevertheless, the effect of hydrocephalus on the behavioural data is not completely clear. Projects on this topic are planed.

Conclusions

Since hamsters showed stereotypic wire gnawing in cage sizes from 1,800 cm^2 until 10,000 cm^2 the welfare of the hamsters even in the very large cages must have been impaired. Frequency and duration of wire gnawing significantly decreased with increasing cage size. Thus, the welfare of pet golden hamsters might be improved by providing enriched cages of at least 10,000 cm^2. Further investigations should follow on the behaviour and development of stereotypic wire gnawing of golden hamsters in differently enriched cages.

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3 CAN WE TELL HAMSTERS ARE STRESSED BY MEASURING THEIR CORTISOL LEVELS?

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CAN WE TELL HAMSTERS ARE STRESSED BY MEASURING THEIR CORTISOL LEVELS?

Sabine G. Gebhardt-Henrich, Katerina Fischer, Andrina R. Hauzenberger, Petra Keller, Andreas Steiger

Vetsuisse Faculty Bern, Institute of Animal Genetics, Nutrition and Housing, Division of Animal Housing and Wlefare, P.O. Box, CH-3001, Bern, Switzerland

Correspondent author:
Sabine G. Gebhardt, Vetsuisse Faculty Bern, Institute of Animal Genetics, Nutrition and Housing, Division of Animal Housing and Welfare, Bremgartenstrasse 109a, P.O. Box, CH-3001 Bern, Switzerland,
Phone: +41-31-631-23-66, Fax: +41-31-631-26-40
e-mail: sabine.gebhardt@itz.unibe.ch

Abstract

The serum levels of corticosterone, cortisol, and ACTH were measured in male and female golden hamsters under different housing conditions. In males, the duration of handling until blood was taken (4.6 min. on average) significantly influenced the concentrations of corticosterone, cortisol, and the ratio of cortisol/corticosterone. Handling times for females were only 2.3 minutes on average and no time effects were found. However, significant effects of series were detected in both sexes. No significant differences in the hormone levels were found due to housing treatments. Values of these hormones in the literature reveal large variation in this species. Due to the sensitivity of hormonal measurements to (sometimes unknown and unavoidable) environmental factors interpretations of the stress levels of golden hamsters based on these hormones must be made with caution.

Keywords: cortisol, corticosterone, ACTH, stress, golden hamster

Introduction

Concentrations of glucocorticoids are commonly used to infer the stress condition in various species (see review by Buchanan, 2000). Unfortunately, poor repeatabilities of the measured values and contradictory results of several studies have raised controversies about the value and applicability of the measurements of these hormones (Rushen, 1991; Sandoe and Simonsen, 1992; Mason and Mendl, 1993). Several problems probably contribute to the difficulties of interpreting hormonal measurements in regard to stress and these have been sufficiently discussed elsewhere (Rushen, 1991; Buchanan and Goldsmith, 2004). In this note we want to report experimental influences on hormonal measurements in golden hamsters in reference to published results on these species and to discuss the difficulties of measuring the stress condition in male and female golden hamsters.

Methods

Animals and husbandry

Forty-five male and sixty female golden hamsters (progeny of Crl: LVG (SYR) from Charles River, Germany) were used in two experiments. In the experiment with male hamsters, animals were assigned to three groups, differing in bedding depth (b_{deep} = 80 cm, b_{medium} = 40 cm and b_{low} = 10 cm of bedding). In the experiment with female hamsters, animals were kept in four different cage sizes (1,800 $cm^{2,}$ 2,500 cm^2, and 5,000 cm^2, 10,000 cm^2). Due to space limitations the experiments were performed at three different times. Additional hamsters for the validation of the ACTH test were kept in cages of 5,000 cm^2. Food and water were offered ad lib. Lighting was artificial with 12h light 12 h dark. Temperatures were between 20 and 26º C. After weaning at around 4 weeks of age, the hamsters were placed into the experimental groups and kept singly for 13 weeks. More details of the experiments are given in Fischer et al. (in preparation) and Hauzenberger et al. (in preparation). Thirteen weeks after weaning hamsters were decapitated after isoflurane-anaesthesia (5%). The duration of catching the hamster out of its cage, the duration of anaesthesia, and the total time from first disturbance to decapitation (= total duration of blood sampling) were recorded. The trunk blood was used for analyses of corticosterone, cortisol, and ACTH. Hamsters of each series and each sex were euthanized on two consecutive days.

An additional ACTH-challenge test was done with 12 adult female golden hamsters for further validation of the cortisol and corticosterone levels. The animals were kept in 5,000 cm^2 cages with 10 cm of bedding. After an i.p. injection of ACTH (Synacthen® 60 µg / 100 g body mass) the anaesthesia and decapitation was done in ascending time intervals. Blood was collected in EDTA-vials.

The experiments were approved by the Cantonal Office of Agriculture and Nature to adhere to the Swiss legislation of housing, anaesthesia, and euthanization of laboratory animals.

Hormonal analyses

After collection blood was centrifuged and the serum was stored in -80º C until it was shipped to the laboratory on dry ice.

The following analyses were done at the Institute of Endocrinology of the Tierärztliche Hochschule Hannover (1, 2) and the Alomed Laboratory in Radolfzell (3) in Germany:

Corticosterone was assayed with the commercial RIA for rats (DPC). Intra-assay variability was 4.3 %, inter-assay variability was 5.8 %.

Cortisol was assayed with in-house RIA (3H), the precision was 1.0 ng/ml. The antibody used was Anti-Cortisol-3-(CMO-) BSA Antiserum (rabbit), for cross-reactions see Klein et al. (1989). Intra-assay variability was 9.2 %, inter-assay variability was 10.9 %.

ACTH was assayed with a chemoluminescence-immunometric assay (Nichols Institute Diagnostics), which was validated for dogs (Schwedes and Müller, 2000). The analytical sensitivity was 2 pg / ml. Intra-assay variability was 7.2 %, inter-assay variability was 8 %.

Statistics

Analyses were done using NCSS® and SAS®. Data and residuals were checked for normality and transformed if necessary, or non-parametric tests were used (see text). All correlation coefficients are Spearman's (r_s). The influences of the duration of handling the hamsters before euthanasia were analyzed by stepwise regressions. Post-hoc multiple comparisons were done using the Tukey-Cramer method (NCSS®).

Results

Males had generally higher values of the measured hormones (Tab. 1). None of the treatment effects (cage size or depth of bedding) influenced the concentration of glucocorticoids although significant differences in behaviour and relative weights of organs were detected (Fischer et al., in preparation, Hauzenberger et al., in preparation).

Tab. 1 Hormonal measurements of male and female golden hamsters. The means are given with the standard deviations in parentheses.

hormone	males N = 44	females N = 57
corticosterone [ng/ml]	32.228 (±18.129)	7.338 (±4.583)
cortisol [ng/ml]	18.340 (±28.141)	8.329 (±6.544)
cortisol/corticosterone	0.511 (±0.515)	1.531 (±1.698)
ACTH [pg/ml]	39.512 (±36.514)	12.714 (±13.520)

Hormones

Corticosterone

In males, which were held in three different depths of bedding, corticosterone levels differed significantly between series (Kruskal Wallis: $\chi^2 = 9.411$, $P = 0.009$). Nine out of 45 values were above a level of 50 ng/ml. All but one high value derived from series 1. Corticosterone was significantly correlated with the duration of catching ($r_s = 0.314$, $P = 0.040$), duration of anaesthesia ($r_s = 0.367$, $P = 0.016$) and total duration until blood samples were taken ($r_s = 0.382$, $P = 0.012$). In a stepwise regression log-

transformed corticosterone levels were significantly correlated with the duration of anaesthesia (partial $r^2 = 0.14$, $F = 7.10$, $P = 0.01$).

Similarly in females in different sized cages, series differed significantly (GLM-ANOVA: $F = 5.92$, $N = 56$, $P = 0.005$). The level of corticosterone was positively correlated with the series (Spearman rank correlation coefficient (r_s) = 0.397, N=56, P=0.002). On day 1 of blood sampling values were higher than on day 2 (GLM-ANOVA: $F = 4.52$, $N = 57$, $P = 0.039$).

Cortisol

In males, cortisol levels were approximately normally distributed (around 18 ng/ml) with the exception of eleven deviating values above a level of 20 ng/ml (Tab. 1). One value was even 8 fold above the mean value. All but two high values derived from series 1, 10 out of 15 hamsters of series 1 had levels above 20 ng/ml. Cortisol levels were significantly higher in series 1 than in series 2 and 3 (Kruskal WallisTest: $\chi^2 = 25.614$, $P < 0.005$). Cortisol levels were significantly correlated with the weight of the epididimal glands ($r_s = 0.317$, $P = 0.043$). In a stepwise regression log-transformed cortisol levels correlated significantly with the duration of catching (partial $r^2 = 0.41$, $F = 28.52$, $P < 0.0001$) (Fig. 1).

In females, there were 14 very high values which were distributed over all three series. No reasons for the outliers could be found. To fulfil the assumption of normality data were log transformed. Cortisol values tended to be higher on day 1 compared with day 2 (GLM-ANOVA: $F = 3.23$, $N = 56$, $P = 0.079$). On day 1 cortisol was negatively correlated with the order of sampling ($r_s = -0.456$, $N = 28$, $P = 0.015$) (Fig. 2).

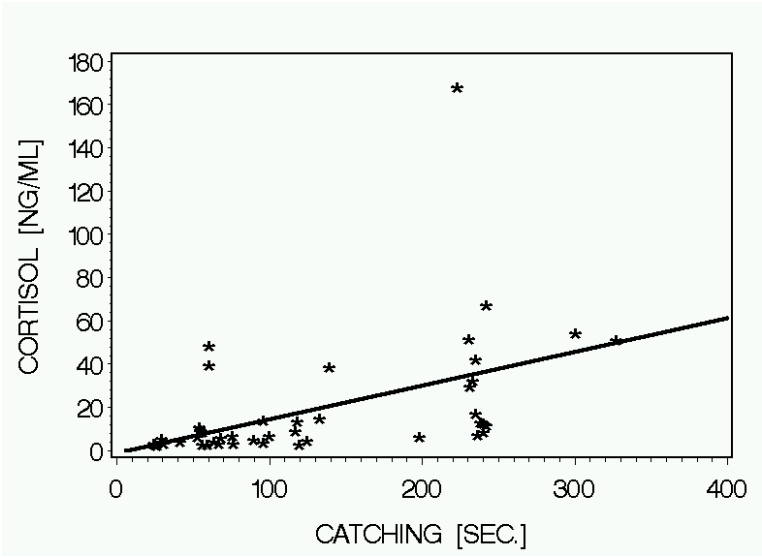

Fig. 1 The concentration of cortisol correlated with the duration of catching (shown for males).

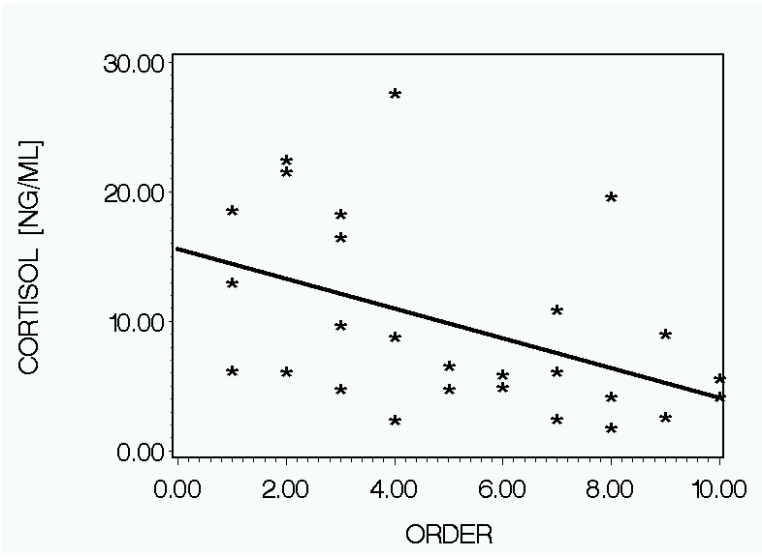

Fig. 2 The concentration of cortisol in females was negatively correlated with the order of sampling on day 1.

Cortisol/corticosterone ratio

In males, the cortisol/corticosterone ratio was significantly correlated with the total duration until blood samples were taken (partial r^2= 0.42, F = 29.97, P < 0.0001) (all data log-transformed) (Fig. 3).

In females the cortisol/corticosterone ratio was negatively correlated with the series (r_s = -0.303, N = 54, P = 0.026).

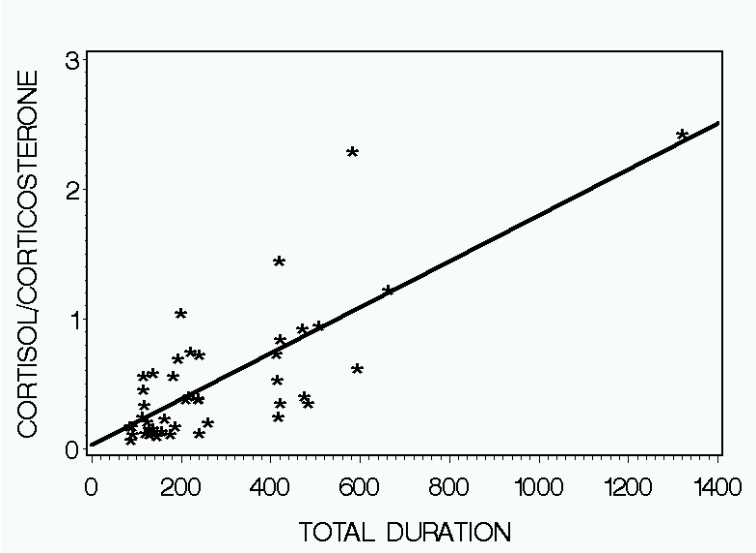

Fig. 3 In males the ratio of cortisol/corticosterone was correlated with the total duration of blood sampling.

ACTH

Levels of ACTH were significantly higher in male hamsters from cages with 80 cm deep bedding compared with male hamsters in low cages (Kruskal Wallis: χ^2=11.319, P<0.005, post hoc critical value of the comparison = 4.723). There were no significant differences among series.

In female hamsters, ACTH (log-transformed) was negatively correlated with the duration of anaesthesia (partial r^2 = 0.13, F = 5.96, P = 0.019).

Thirteen values of females were too small for measurement (< 2pg/ml, 10 of them in series 3).

Sampling effects

In the first series it took longer to catch the male hamsters out of their cages than in the following series (Kruskal-Wallis: χ^2=25.409, P<0.005, post hoc critical value= 4.723). Anaesthesia was also significantly longer in series 1 (Kruskal-Wallis: χ^2=31.050, P<0.005, post hoc critical value=4.723), as was total duration of blood sampling (Kruskal-Wallis: χ^2=28.282, P<0.005, post hoc critical value=4.731). Most time was needed to catch hamsters in deep bedding cages of 80 cm (Kruskal Wallis: χ^2=5.757, P=0.05).

The duration of catching and the duration of anaesthesia were significantly correlated (r_s =0.674, P<0.005).

In females there were no significant differences among series of the duration of catching, anaesthesia, or total duration of blood sampling (all P values > 0.4). There was a trend that the duration of anaesthesia and total duration of blood sampling were higher on day 1 than on day 2 although just not significant (Kruskal-Wallis Test for total duration of blood sampling: χ^2=3.552, P=0.06). As expected, it took significantly more time to catch hamsters out of the largest cage than out of the

smaller cages (GLM-ANOVA: F=9.00, N=56, P=0.00007, post hoc critical value=4.634, P<0.01) (Fig. 4). This resulted in a higher total duration of blood sampling in the largest cages (Kruskal Wallis Test: 10.068, N=56, P=0.018) compared with the other cage sizes (post hoc critical value=4.895, P<0.05).

It took more than twice as long to catch the male hamsters out of their cages than the female hamsters (Tab. 2).

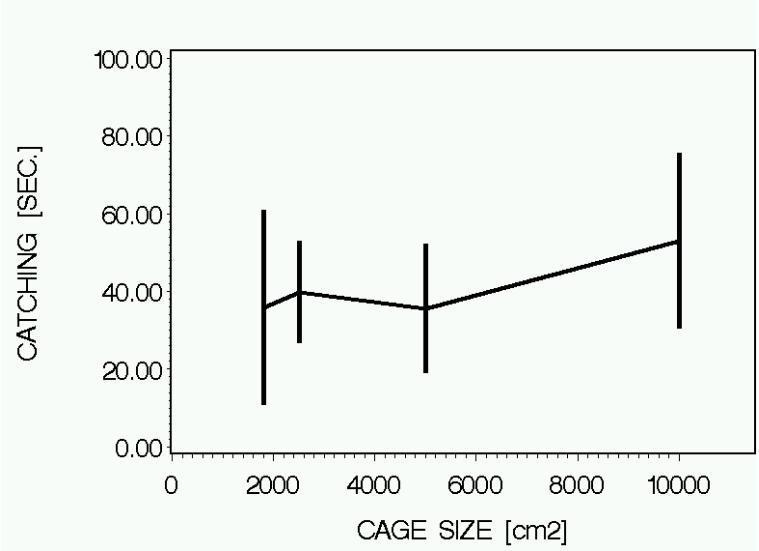

Fig. 4 It took longer to catch hamsters out of the largest cage.

Tab. 2 Durations [min.] of catching, anaesthesia, and the total time male and female hamsters. Standard deviations are given in parentheses. NS – not significant

Variable	male	female	χ^2	P-Value
catching	2.1 (1.46)	0.68 (0.20)	37.90	< 0.0001
anaesthesia	2.4 (2.75)	1.6 (0.56)	1.49	NS
total duration	4.6 (3.69)	2.3 (0.65)	15.24	< 0.0001

ACTH-Challenge Test

In the ACTH-challenge test there was an elevation of cortisol 3 minutes after the injection of ACTH with a maximum value of 100 ng/ml 20 minutes after injection (Fig. 5). Corticosterone values peaked 1 hour after the injection with a maximum of 53 ng/ml (Fig. 5).

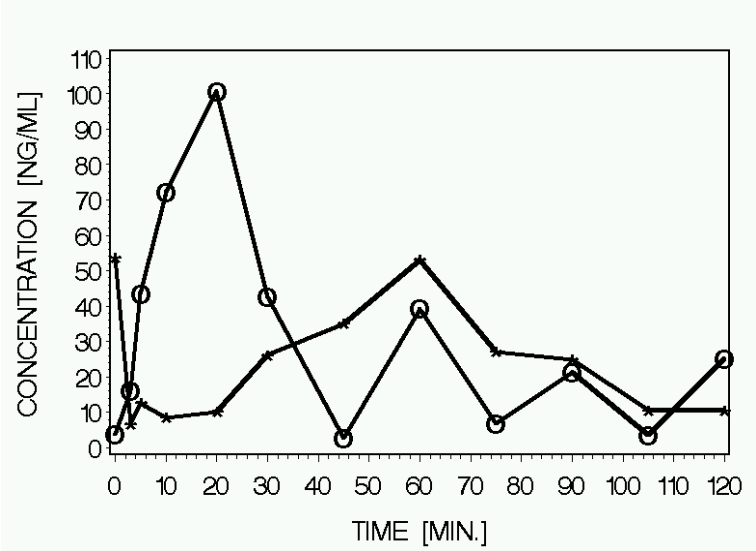

Fig. 5 ACTH challenge experiment, circles: cortisol, stars: corticosterone.

Discussion

Various concentrations of cortisol in golden hamsters under different (stress) situations have been published (App. VI). Possible causes of the differences in the hormonal reaction to stress have been directly investigated, namely age, different stressors ((Wommack and Delville, 2003; Taravosh-Lahn and Delville, 2004), duration (Ottenweller et al. 1985), time of day (Albers et al. 1985), and experience (Weinberg and Wong, 1986). Different methods could explain the discrepant values. However, it is not clear why the studies of Wommack and Delville (2003) and Taravosh-Lahn and Delville (2004) obtained such different values under the same stress with the same hormonal analyses. Unknown influences varying with time might be very important and the factor age might be confounded with the factor time.

Different aged individuals should be tested and their blood taken at the same time to attribute effects clearly to the age. Our cortisol concentrations in males could indicate that they were acutely stressed at the time of blood sampling. This is supported by the result that corticosterone, cortisol, cortisol/corticosterone ratio and ACTH mainly depended on how much time was spent from first disturbance of the hamsters until blood samples could be taken after euthanasia. It shows the sensitivity of the pituitary-adrenal axis (PAA). Cortisol was already elevated after three minutes of the injection of ACTH. In some cases blood samples probably where taken at a point when hormone levels had already begun to increase. On average, blood samples in males were taken about 4 minutes after the first disturbance. Since the duration of catching the hamsters depended on the treatments (bedding depth or cage size) we cannot draw any conclusions concerning the state of stress in the hamsters in relation to their treatments. The decrease of ACTH with time can be explained by the

negative feedback of cortisol: increasing cortisol levels suppress the release of ACTH in the pituitary gland.

ACTH seemed to increase with increasing duration of sampling, although not significantly. It is the first hormone that is released in a stress situation and increases within a few seconds until a negative feedback of the glucocorticoids starts. Because of the immediate reaction it is not possible to draw any conclusions about the stress level. In series 4 we measured 13 values below 2 pg/ml. The reason for such small values could be the fast decomposition of ACTH due to the negative feedback, which does not explain why it occurred so frequently in series 3.

Our data show that sampling blood for measuring chronical stress must be taken quickly, at least within 2.5 minutes, before levels rise due to the acute stress of sampling. This might be difficult because the (Swiss) Animal Welfare Legislation forbids decapitation without previous anaesthesia. For more accurate values hamsters could be administered with a catheter, yet it is very difficult in this species and causes stress itself. There is no solution for cages with deep bedding, for the time until hamsters are caught out of their cages can take just as long as the PAA system needs to react to acute stress.

Faecal samples have their advantage in being non-invasive, a critical drawback is that it gives only average hormone levels over a, sometimes unknown, period and this method is sometimes not validated (Buchanan and Goldsmith, 2004). During the ACTH-challenge test no increase in suspected metabolites of cortisol was found in the faeces (unpublished data).

Blood samples were taken on two following days. Although the duration of anaesthesia and total duration of sampling did not differ significantly among days of sampling, there was a tendency of higher values of total duration of sampling on day 1. Corticosterone levels on day 2 were lower than on day 1. It is likely that hamsters

were disturbed from sampling on the previous day, while sleeping in the bedding, but because of a habit effect PAA-reaction was lower on the second day which resulted in lower corticosteroid values. So it might be better to perform blood sampling within one day. However, further complications arise due to the dependence of the PAA on chronobiology. When treatments affect the circadian rhythms as it was found (Hauzenberger et al., in preparation), then it would be impossible to relate certain hormone levels to circadian times.

Other complications could arise from a shorter duration of catching due to the training of the handler. It means that hamsters that were caught later were caught faster. Although it seems to have had no general influence in our analyses, we cannot exclude an effect, since on day 1 hamsters that were caught later had a lower cortisol level. This could be due to the time of day, or due to a difference in duration of anaesthesia and total duration of blood sampling.

The correlation between the duration of catching and the anaesthesia was probably due to the level of experience. Furthermore, the equipment for anaesthesia was not working properly in the first series which might have contributed to the effect of series. Since the experiments were done year-round, an effect of the season cannot be excluded.

Conclusions

Since the stress response of the PAA is very sensitive to many unwanted and uncontrollable factors the results have to be interpreted with caution.

Physiological measures of animal welfare can only serve as an additional brick stone to accompany parameters such as behaviour. No parameter can be interpreted by itself, but always in the context of additional findings.

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4 Appendix

App. I Cage size 1 (right): 1,800 cm^2 and cage size 2 (left): 2,500 cm^2

App. II Cage size 3: 5,000 cm^2

App. III Cage size 4: 10,000 cm^2

App. IV Behaviour catalogue. Description of behavioural codes for analyses of video-tapes with Observer™ *Version 5.0* (Noldus). *Analysis of rearing was made for rearing including rearing on the wire and separately, after subtraction of rearing on the wire. **Summation was calculated after Observer-analyses.

Behaviour	***Description***
wheel-running	Use of running wheel
running	Horizontal locomotion
resting	Standing on all four limbs, resting, includes sniffling and stretching
rearing	Rise of at least one limb, standing on hind legs. Includes rearing on the wire which means that one ore two legs were put on the wire. Rearing on the wire occurred often between wire gnawing bouts.*
climbing	Climbing at the wire, no contact to the floor, resting in a climbing position at the wire, hanging at the wire top
gnawing	Gnawing at twigs, cardboard box, wooden house, hey or straw
wire gnawing	Gnawing at bars or at the water bottle, bouts were frequently interrupted by rearing at the wire.
grooming	Grooming and scratching movements or wallowing in the sand bath
feeding	Dealing with food, sniffling at food, eating food, filling cheek pouches with food
digging	Digging trough the litter, pawing in the litter or the sand, but also pawing on the seam of the plastic bowl.
drinking	Mouth on the front of the water bottle tube, not gnawing.
unknown	The behaviour of the hamster could not be determined because the hamster was hidden or visibility was poor
Location	***Description***
running-wheel	Any activity inside the wheel.
running wheel frame	Sitting, resting or climbing on the frame of the wheel. Hamster is always in sight
behind running-wheel	Hamster is not in sight, behind the wheel, between wheel and wire.
on running-wheel**	Summation of the time spent behind the wheel and on the frame of the wheel
sand bath	The hamster has at least one pair of limbs in the sand bath
wire	Any activity during which the hamster has at least one limb on the wire.
water bottle	Drinking or gnawing on the water bottle
food bowl	The hamster has at least one pair of limbs in the food bowl
on house	The hamster has at least one limb on the house
open space	The hamster is somewhere in the open space between the other locations, not in the depth
depth	The hamster is hidden in the depth of the litter, or is digging in the litter while some body parts are hidden in the litter.
corner behind wheel	The hamster stays in the corner between the wheel and the wire. This is a dark place where hamsters are hidden. The behaviour analysis is not always possible.
location unknown	If location is not determinable.

App. V Number of hamsters that were observed wire gnawing in each cage size (χ^2_3 = 2.06, P = 0.5581)

Cage size [cm^2]	Wire gnawing	No wire gnawing	Total
1,800	10	5	15
2,500	6	8	14
5,000	7	8	15
10,000	7	8	15
Total	**30**	**29**	**59**

App. VI Concentrations of cortisol [ng/ml] (STD) in golden hamsters under control conditions and various stress treatments

year	control	stress	sex	kind of stress	author	method
2004	1.3	2.6	both	conspecific intruder, clean cage, day 27	Taravosh-Lahn and Delville, 2004	EIA
2004	2 – 2.5	1.5 – 2.1	both	conspecific intruder, clean cage, day 41	Taravosh-Lahn and Delville, 2004	EIA
2003	4.5	10	male	conspecific intruder, day 28	Wommack and Delville, 2003	EIA
2003	4.5	14	male	clean cage, day 28	Wommack and Delville, 2003	EIA
2003	6	14	male	conspecific intruder, day 42	Wommack and Delville, 2003	EIA
2003	6	5	male	clean cage, day 42	Wommack and Delville, 2003	EIA
2003	12.7 (4.47)	20.56 (6.01)	male	weasel odor	Zhang, Cao, Gao, Yang, Sun, Zhang, and Wang, 2003	RIA
2001	38	65 - 70	male	conspecific intruder	Jasnow, Drazen, Huhman, Nelson, and Demas, 2001	RIA
1998	1.8	3.7	male	cage size	Kuhnen and Werner, 1998	?
1986	8	26 - 38	male	new cage	Weinberg and Wong, 1986	RIA
1986	9.6	18	female	new cage	Weinberg and Wong, 1986	RIA
1985	2.5 - 18				Albers, Yogev, Todd, and Goldman, 1985	RIA
1985	4	60	male	supine restraint	Ottenweller, Tapp, Burke, and Natelson, 1985	RIA
1972	5.9 (1.5)	6.1 (1.5)	male	conspecific intruder	Brain, 1972	Protein Binding assay
1972	12.8 (2.6)	11.9 (1.9)	female	conspecific intruder	Brain, 1972	Protein Binding assay
1970	4.5 (0.4)	62.4 (0.74)	male	ether stress	Gaskin and Kitay, 1970	Protein Binding assay
1970	3.8 (0.9)	24.7 (5.1)	female	ether stress	Gaskin and Kitay, 1970	Protein Binding assay

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