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Clinical pathology and cardiovascular parameters are not influenced by housing rats under increased environmental complexity

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Abstract

Since the release of the revised Appendix A from the Council of Europe for housing of laboratory animals there have been claims that laboratory animals should be housed under more complex conditions; known popularly as enrichment. A number of studies have expressed concerns that this may increase uncontrollable variation in the animals, thereby creating the need for greater numbers of animals. Within neurobiology there would appear to be a scientific basis for such concern. However, even though this may be used as an argument for denying the animal environmental enrichment, it is unclear whether there is any basis for concern within other research areas. The aim of this study, therefore, was to explore whether clinical pathology and cardiovascular parameters were influenced by housing rats under environmentally enriched conditions. Male, Sprague-Dawley rats were housed under three different regimes: non-enriched, standard-enriched (according to the guidelines of the Council of Europe) and extra-enriched with a shelf and higher cages. All housing forms were based upon commercially available, standardised equipment. A total of 41 different parameters were monitored via clinical pathology, telemetry and coagulation tests and virtually no differences were observed in relation to the manner in which the rats were housed. The uncontrollable variation observed in our study was compared to within-strain variation data supplied from the breeder and was relatively low in all three types of housing. We conclude, based upon our studies in male, Sprague-Dawley rats, that so far there is no basis for concern that enriched housing will lead to increased group sizes when using animals for research within this field and, as such, there is no reason not to enrich the environment of such rats.

Keywords: *animal welfare, clinical pathology, environmental enrichment, housing conditions, rats, telemetry*

Introduction

Since 1959, there has been much focus on reduction, refinement, and replacement as key principles for the use of animals in research (Russell & Burch 1959). The practice of providing animals under captive care, complex housing conditions and environmental items to stimulate physical activity and natural behaviours as well as reduce stereotypic behaviours is more popularly referred to as environmental enrichment. Environmental enrichment has been defined as an improvement in the biological functioning of captive animals resulting from modifications to their environment (Newberry 1995) and may have a positive impact on well-being (Sørensen *et al* 2004), and is therefore an important form of refinement. The enrichment can either involve structuring of components within the primary enclosure; cage furniture, objects for manipulation, or cage complexities — or social contact and communication among members of the same species, although it can also include non-contact communication among individuals through visual, auditory, and olfactory signals (Institute of Laboratory Animal Resources 1996). Enrichment is increasingly appreciated as a way of improving the well-being of rodents, providing them with opportunities for species-specific behaviours that might be available to them in the wild (Ottesen *et al* 2004; Smith & Corrow 2005), and is seen as bringing crucial features of the environment into the laboratory to allow expression of natural behaviours (Blanchard & Blanchard 2003). Environmental enrichment should be regarded both as an essential component of the overall animal care programme; equally as important as nutrition and veterinary care (Baumans 2005), and recently the revised Appendix A of the Council of Europe (CoE) Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe 2006) stated that environmental enrichment should generally be provided unless withholding is justified on veterinary or welfare grounds (Hansen *et al* 2002; Stauffacher *et al* 2002).

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Several authors, however, state that little is known about the influence of environmental enrichment on biological parameters (Hutchinson *et al* 2005), and highlight how future research on environmental enrichment would benefit from improved knowledge of the functions of behaviour performed in captivity and more rigorous experimental design (Newberry 1995) and that better documentation of environmental variables and their correlation with experimental results is needed to gain critical knowledge of the relationship between an animal's environment, its well-being, and science (Weed & Raber 2005), eg the effect on physiological parameters and the impact on scientific outcome (Baumans 2005). There have been concerns that environmental enrichment might jeopardise the standardisation of experiments by increasing uncontrollable variation (van de Weerd *et al* 2004) leading to the need for greater numbers of tests animals, especially within behaviour and neurobiology. For example, a study in DBA/2J mice showed that the effects of enrichment designs were not consistent, but varied according to sex and the variables studied and that enrichment led to enhanced variation in physiological traits, open-field and food-drive tests (Tsai *et al* 2003). On the other hand, it has been stated that animal welfare can be improved by beneficial enrichments without disrupting standardisation (Würbel & Garner 2007) and, for example, no effect on the variation was shown in another behavioural study in BALB/c and C57BL/6 mice (Augustsson *et al* 2003), or in a similar Sprague-Dawley rat study (Sørensen *et al* 2010). It has been further concluded that the risk of increased variability is not particularly well documented (Sørensen *et al* 2004), and probably too small to affect the number of animals needed (Eskola *et al* 1999), or that the choice of statistical method to analyse variation, rather than providing environmental enrichment, may influence the interpretation of inter-individual variability (Augustsson *et al* 2003). Controversially, it has been argued, on the other hand, that enhanced welfare through successful enrichment programmes reduces the number of animals needed as fewer animals may be lost throughout the course of experiments (van de Weerd *et al* 2002). Furthermore, that enrichment may actually improve validity, reliability, and replicability by reducing the number of abnormal animals introduced into experiments (Garner 2005), and that the housing conditions of laboratory mice can be markedly improved without affecting the standardisation of results (Wolfer *et al* 2004). It would appear that there is no simple way of predicting the effects of environment on uncontrollable variation since the effects seem to be time-, place-, animal- and parameter-dependent, but with adequate research techniques, designs, and standardisations some degree of experimental variation can be controlled but not totally eliminated (Mering *et al* 2001), but also that standardisation is a flawed concept, which entails the risk of obtaining results of poor external validity and therefore needs to be profoundly revised (Würbel & Garner 2007).

It should certainly be considered that environmental enrichment has important effects on brain morphology (Bayne 2005) and structural and biochemical changes in the brain that correlate with improved learning and memory (Schrijver *et al* 2004), which primarily accelerate habituation to novelty and improved spatial learning and memory, but with no altered basal and response levels of plasma ACTH and corticosterone (Schrijver *et al* 2002). Environmental enrichment also increases the number of synapses per neuron in the visual cortex (Jones & Greenough 1996) which, without enhancing well-being, may be detrimental to the research for which the animals are used (Benefiel *et al* 2005).

It is still unclear the extent to which other research parameters are influenced by enrichment, and whether there is any argument to withhold enrichment from animals not involved in behavioural and neurobiological research, and it has even been stated that environmental enrichment is a cause of, rather than a cure for, poor reproducibility of experimental outcomes (Richter *et al* 2009). The aim of this study, therefore, was to evaluate the possible influence on basal clinical pathology and cardiovascular parameters on rats housed under non-enriched conditions compared to the enrichment demanded by the revised Appendix A (Hansen *et al* 2002) and to conditions of further enrichment.

Materials and methods

A total of 149, male, outbred Sprague-Dawley (NTac:SD) rats (*Rattus norvegicus*) (Taconic, Lille Skensved, Denmark) were used in all studies. The rats were socially housed with four animals per cage for the clinical chemistry, haematology and coagulation studies and pair housed for the telemetry study at the research facility for between eleven and fourteen weeks from the age of three-to-four weeks post weaning. The actual study initiation after housing in one of the three standardised housing conditions described below was either blood sampling in the clinical chemistry and haematology study, telemetry recording of blood pressure and heart rate, or anaesthesia and tail bleeding in the coagulation study.

The rats were housed under three different, standardised housing conditions: (i) non-enriched housing conditions (Figure 1) in a standard Type IV macrolon cage, 595 × 380 × 200 mm (length × breadth × height), (Scanbur A/S, Karlslunde, Denmark) with aspen bedding (Tapvei, Kortteinen, Finland); (ii) standard-enriched housing conditions (Figure 2), according to the revised Appendix A of the CoE Convention, ETS 123 in a standard Type IV macrolon cage, 595 × 380 × 200 mm (length × breadth × height) (Scanbur A/S, Karlslunde, Denmark) with aspen bedding (Tapvei, Kortteinen, Finland), paper-based nesting material, Enviro-Dri® (Lillico, Surrey, UK), a Novo Nordisk hide (Repsol, Brønderslev, Denmark), 20 × 14 cm (length × breadth) and placed at a height of 12 cm, and an aspen brick, size M (100 × 20 × 20 mm; length × breadth × height) (Tapvei, Kortteinen, Finland); and (iii) extra-enriched housing conditions (Figure 3) in a Scantainer NOVO type IV cage, (595 × 380 × 325 mm; length × breadth × height), with a built-in shelf, (Scanbur A/S, Karlslunde, Denmark), aspen bedding (Tapvei, Kortteinen, Finland), paper-based nesting material, Enviro-

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Dri® (Lillico, Surrey, UK), a Novo Nordisk hide (Repsol, Brønderslev, Denmark), 20 × 14 cm and placed at the height of 12 cm and an aspen brick, size M, biting stick (Tapvei, Kortteinen, Finland).

All cages were changed bi-weekly at which time the nesting material, hide and biting stick were transferred to the new, clean cage. The rats were housed in a climate-controlled room at 20 (± 2)ºC, 45 (± 10)% relative humidity, 8–15 air changes per hour and 12 h of light from 0600 to 1800h. They were fed *ad libitum* with a commercial rodent standard diet (Altromin, Type 1320, 'Maintenance Diet Rats/Mice', Brogaarden, Gentofte, Denmark) and had access to tap water from an automated watering system (Edstrom Europe, Hereford, UK) that was flushed daily. The animals were observed at least once per day and were in the care of experienced animal technicians.

This study was approved by the Ministry of Justice, Animal Experiments Inspectorate, Denmark.

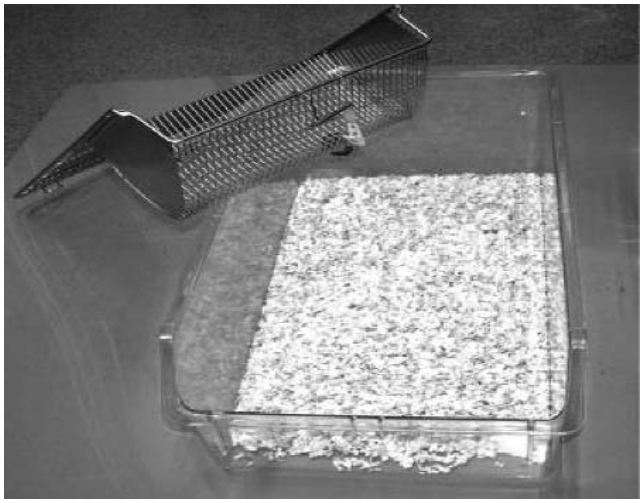
Clinical chemistry and haematology

At the age of 15 weeks (half of each group) and 17 weeks (half of each group), 25, 26 and 26 rats from the non-enriched, standard-enriched, and extra-enriched environments, respectively, were blood sampled from the abdominal aorta under fentanyl/fluanison/midazolam (Hypnorm®, VetaPharma, Leeds, UK; Midazolam, Roche, Hvidovre, Denmark) anaesthesia, 1 ml Hypnorm (0.315 mg fentanyl ml^{-1}; 10 mg fluanison ml^{-1}), 1 ml midazolam (5 mg) plus 2 ml sterile water in a dose of 0.33 ml 100 g^{-1} rat, intraperitoneal, and thereafter euthanised. Blood was sampled in K_2EDTA-prepared tubes and cholesterol, triglycerides, albumin, total protein, calcium, phosphorus, chloride, sodium, potassium, asparagine aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, creatinine kinase, urea, creatinine and glucose were measured by Cobas Mira Plus (Global Medical Instrumentation Inc, Ramsey, Minnesota, USA). Also, fibrinogen C (Fib-C), thrombin time (TT), activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured by ACL 300 (Instrumentation Laboratory SpA, Milan, Italy), while haematological examination was carried out by Advia 120 (Siemens, Ballerup, Denmark) registering white blood cells (WBC), red blood cells (RBC), haemoglobin, haematocrit, erythrocyte mean corpuscular volume (MCV), haemoglobin (MCH) and haemoglobin concentration (MCHC), platelets, reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells (LUC).

Blood pressure and heart rate

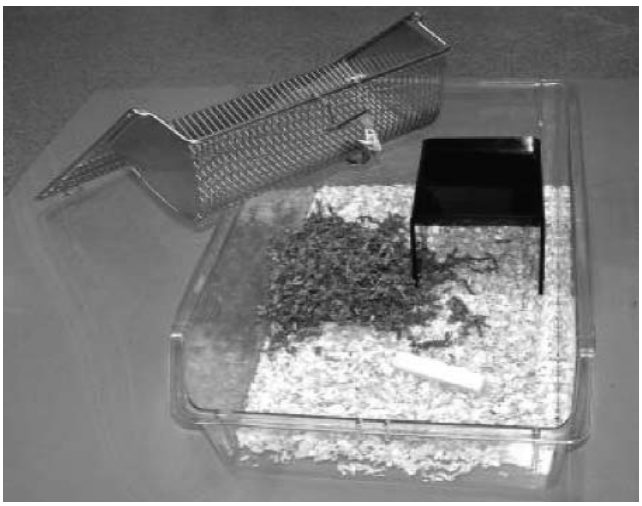
All rats used in the telemetry study were, for welfare reasons, pair housed with a companion rat not participating in the actual study. The rats were divided into three groups of eight pair-housed rats that were each housed in one of the three different housing environments: non-enriched, standard-enriched, and extra-enriched, respectively. On delivery, they were approximately four-weeks old and randomly allocated to one of the three different housing

Figure 1



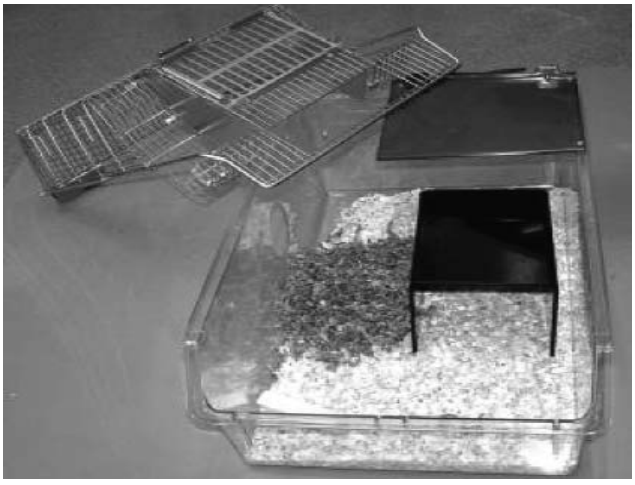
Non-enriched housing conditions used for studying the impact of enrichment on clinical pathology and cardiovascular parameters in rats.

Figure 2



Standard-enriched housing conditions used for studying the impact of enrichment on clinical pathology and cardiovascular parameters in rats.

Figure 3



Extra-enriched housing conditions used for studying the impact of enrichment on clinical pathology and cardiovascular parameters in rats.

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conditions. After an acclimatisation period of between seven and ten weeks, the rats in the telemetry study were anaesthetised with Isoflurane (Baxter A/S, Allerød, Denmark) and implanted intra-peritoneally with a TL11M2-C50-PXT telemetry transmitter (Data Sciences International, St Paul, USA) and with an arterial catheter into one of the femoral arteries. Pre-emptively, they received 0.05–0.1 mg kg^{-1} Temgesic, (Schering-Plough A/S, Farum, Denmark) and 5 mg kg^{-1} Rimadyl Vet (Orion Pharma, Nivå, Denmark) as analgesia plus 0.05 ml 100 g^{-1} Streptocillin Vet 2000.000 IE (Boehringer Ingelheim, København Ø, Denmark) as prophylactic antibiotic treatment. For the following two days they received 5 mg kg^{-1} Rimadyl Vet as post-operative analgesia. All rats were allowed a period of recuperation of at least four weeks, post-operatively and were required to pass a general health check before taking part in the study. In total, rats were housed for 13 weeks in the three different housing conditions before cardiovascular measurements began. The computer programme, Notocord-HEM v 3.5 (Notocord, Croissy Sur Seine, France) was used to acquire and display the haemodynamic signals obtained by telemetry. All rats had their blood pressure and heart rate monitored for five days with 24 h of continuous haemodynamic recording.

Coagulation tests

Forty-eight rats were housed, from the age of weaning, ie approximately three-weeks old, for 12 to 14 weeks under one of three different housing conditions. All rats were weighed once a week and after the pre-study housing period, a tail-bleeding experiment, being a widely used coagulation test within haemostasis and haemophilia research, was performed, as previously described (Lauritzen *et al* 2008). The rats were weighed, and anaesthetised with 50 mg kg^{-1} pentobarbital sodium ip (Veterinærapoteket, University of Copenhagen, Frederiksberg, Denmark). After catheterisation, the tail of the rat was placed in a plastic tube containing 50 ml isotonic saline kept at 37°C in a water bath (TYP V3/8, Julabo, Seelbach, Germany). After five minutes, the 16 animals in each housing group were randomised to receive an intravenous injection of either 200 IU kg^{-1} heparin in 2 ml kg^{-1} (Leo Pharma, Ballerup, Denmark) or isotonic saline (control group). After another ten minutes, tail bleeding was induced by cutting of the outermost 2 mm of the tail with a nail scissor after which the tail was re-positioned in the plastic tube containing saline. The amputated tail tip was weighed after the experiment. Bleeding was observed for 30 min and, thereafter, still anesthetised, the animals were euthanised by an overdose of pentobarbital. Total bleeding time was defined as the cumulated bleeding time over the 30-min observation period, including re-bleedings. Blood loss was determined spectrophotometrically, as previously described (Lauritzen *et al* 2008).

Statistical analysis

The changes in bodyweight over time were tested using repeated measures ANOVA. All clinical chemistry, haematology and coagulation data were tested for normal distribution by Anderson-Darling test. Normally distributed data were described by mean (± SD), differences in their means were compared by a one-way ANOVA and differences in their variances were compared by Bartlett's test. Data sets not following a normal distribution were described by median, maximum and minimum, and differences in their medians were compared by Kruskal-Wallis test and differences in their ranges were compared by Levene's test. For each normally distributed data set the smallest difference, which could be shown to be significant, was calculated and compared between the three different housing environments by Kruskal-Wallis test. This calculation was based upon the coefficient of variance found for three different environments and a group size of 25 animals ($\mu = 0.9$; $\alpha = 0.05$). Telemetric data were analysed by calculating individual AUC's and the difference between groups were tested using ANOVA.

Results

There were no significant differences in bodyweight between the groups of rats housed under the three different housing conditions over the 12-weeks observation period (Figure 4).

In general, the clinical pathology, haematology and coagulation parameters differed very little in relation to the three different housing conditions (Tables 1–6). Rats housed under either standard-enriched or extra-enriched conditions had significantly higher mean albumin than rats housed under non-enriched conditions ($P < 0.01$) (Table 1), while rats housed under non-enriched conditions had significantly higher fibrinogen C level (Table 5) as well as counts of white blood cells and neutrophils (Table 6) than those housed under one of the two other housing conditions. The only significant difference in variation within the clinical chemistry seemed to be a lower variation in the number of red blood cells observed under the non-enriched housing conditions ($P < 0.05$) (Table 6). There were no significant differences between the three different environments when subjecting all normally distributed clinical pathology and haematological parameters to a power analysis (Table 7).

There were no significant differences between the heart rate and blood pressure of rats from the three different housing conditions (Figures 5–6).

No significant differences in tail-bleeding time were found between animals housed under the three different housing conditions regardless of whether they were non-treated or heparin-treated. The coefficient of variance of the bleeding time in the non-treated animals did not vary significantly between the three different housing conditions, whereas the variance in the heparin-treated animals could not be estimated, as most rats were still bleeding by the end of the observation period (Figure 7). Similarly, no significant differences in blood loss were found between animals housed under the three different conditions, neither in non-treated nor in heparin-treated rats. In both the non-treated and the heparin-treated animals, the coefficient of variance of the blood loss was not significantly different between the three different housing conditions (Figure 8).

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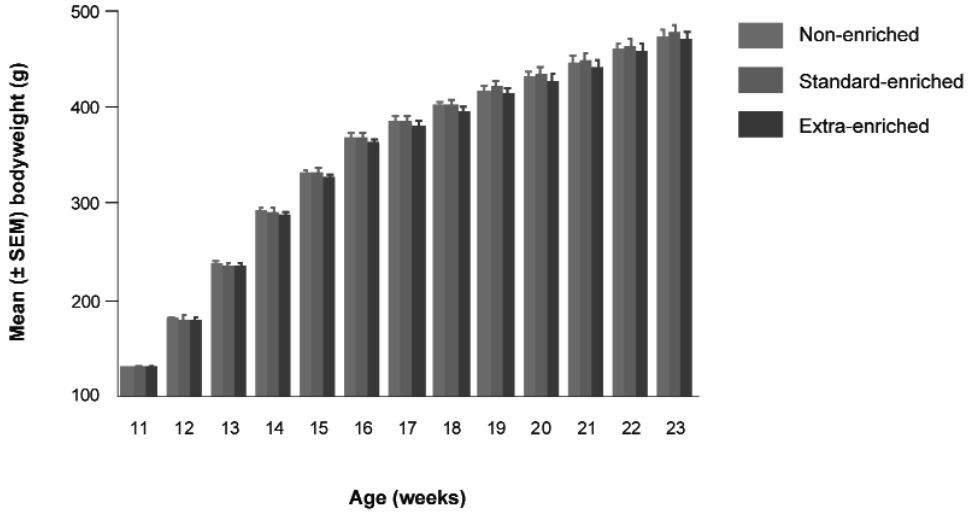
Figure 4



The impact of three different housing environments on the mean (± SEM) bodyweight in rats (n = 16). There were no significant differences between housing environments.

Table 1 The impact of three different housing environments on the mean (± SD) of serum lipids and proteins.

Environment	Parameter	Unit	Mean (± SD)	CV%	Median	Range
Non-enriched (n = 25)	Cholesterol	g L^{-1}	2.25 (± 0.42)	18.7		
	Triglycerides	mmol L^{-1}			1.20	0.33–3.08
	Albumin**	g L^{-1}	28.10 (± 1.05)^{xy}	3.8		
	Total protein	g L^{-1}	57.96 (± 2.8)	4.8		
Standard-enriched (n = 26)	Cholesterol	g L^{-1}	2.13 (± 0.28)	13.3		
	Triglycerides	mmol L^{-1}			1.26	0.80–2.18
	Albumin**	g L^{-1}	28.9 (± 1.1)^{x}	3.9		
	Total protein	g L^{-1}	57.71 (± 3.5)	5.9		
Extra-enriched (n = 28)	Cholesterol	g L^{-1}	2.17 (± 0.38)	17.5		
	Triglycerides	mmol L^{-1}			1.14	0.56–1.84
	Albumin**	g L^{-1}	29.00 (± 1.04)^{y}	3.6		
	Total protein	g L^{-1}	59.6 (± 2.11)	4.0		

Coefficient of variation (CV) is calculated as SD/mean × 100%.
** $P < 0.01$ for differences in means by ANOVA; ^{x} Means/median differ by $P < 0.01$; ^{y} Means differ by $P < 0.01$.

Table 2 The impact of three different housing environments on serum electrolytes.

Environment	Parameter	Unit	Mean (± SD)	CV%	Median	Range
Non-enriched (n = 25)	Calcium	mmol L^{-1}			2.70	1.82–1.84
	Phosporus	mmol L^{-1}	1.89 (± 0.25)	13.3		
	Chloride	mmol L^{-1}			106.0	100–111
	Sodium	mmol L^{-1}			145.0	140–154
	Potassium	mmol L^{-1}			4.1	3.6–5.3
Standard-enriched (n = 26)	Calcium	mmol L^{-1}			2.68	2.51–2.90
	Phosporus	mmol L^{-1}	2.01 (± 0.30)	14.9		
	Chloride	mmol L^{-1}			107.0	101–112
	Sodium	mmol L^{-1}			145.0	140–152
	Potassium	mmol L^{-1}			4.1	3.9–5.1
Extra-enriched (n = 28)	Calcium	mmol L^{-1}			2.65	2.50–2.76
	Phosporus	mmol L^{-1}	1.97 (± 0.25)	12.9		
	Chloride	mmol L^{-1}			106.0	102–110
	Sodium	mmol L^{-1}			145.0	142–147
	Potassium	mmol L^{-1}			4.1	4.7–4.8

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Table 3 The impact of three different housing environments on serum enzymes.

Environment	Parameter	Unit	Median	Range
Non-enriched (n = 25)	Asparagine aminotransferase	U L^{-1}	77.5	55–182
	Alanine aminotransferase	U L^{-1}	63.0	39–146
	Lactic dehydrogenase	U L^{-1}	167.0	76–699
	Alkaline phosphatase	U L^{-1}	126.0	91–1,032
	Creatine kinase	U L^{-1}	204.0	95–445
Standard-enriched (n = 26)	Asparagine aminotransferase	U L^{-1}	94.0	67–421
	Alanine aminotransferase	U L^{-1}	72.5	48–482
	Lactic dehydrogenase	U L^{-1}	217.5	100–2,484
	Alkaline phosphatase	U L^{-1}	129.0	81–1,652
	Creatine kinase	U L^{-1}	169.0	114–424
Extra-enriched (n = 28)	Asparagine aminotransferase	U L^{-1}	81.5	59–308
	Alanine aminotransferase	U L^{-1}	65.5	45–313
	Lactic dehydrogenase	U L^{-1}	164.5	80–1,280
	Alkaline phosphatase	U L^{-1}	122.5	80–1,917
	Creatine kinase	U L^{-1}	155.5	94–352

Table 4 The impact of three different housing environments on serum urea, creatinine and glucose.

Environment	Parameter	Unit	Mean (± SD)	CV%	Median	Range
Non-enriched (n = 25)	Urea	mmol L^{-1}	6.67 (± 0.70)	10.4		
	Creatinine	µmol L^{-1}			43.50	31.0–65.3
	Glucose	mmol L^{-1}			10.31	7.66–19.70
Standard-enriched (n = 26)	Urea	mmol L^{-1}	7.14 (± 0.71)	9.89		
	Creatinine	µmol L^{-1}			46.55	38.2–81.5
	Glucose	mmol L^{-1}			10.47	6.74–22.78
Extra-enriched (n = 28)	Urea	mmol L^{-1}	6.8 (± 0.66)	9.64		
	Creatinine	µmol L^{-1}			45.95	38.4–63.9
	Glucose	mmol L^{-1}			10.52	8.84–15.08

Table 5 The impact of three different housing environments on the mean (± SD), median and range of coagulation parameters: fibrinogen C (Fib-C), thrombin time (TT), activated partial thromboplastin time (APTT) and prothrombin time (PT) of rats.

Environment	Parameter	Unit	Mean (± SD)	CV%	Median	Range
Non-enriched (n = 25)	Fib-C***	g L^{-1}	1.71 (± 0.11)^{z}	6.5		
	TT	s	38.08 (± 3.20)	8.4		
	APTT	s			20.9	15.9–34.2
	PT	s			15.0	12.9–16.0
Standard-enriched (n = 26)	Fib-C***	g L^{-1}	1.59 (± 0.08)^{x,z}	4.3		
	TT	s	38.32 (± 2.22)	5.8		
	APTT	s			21.2	12.9–33.2
	PT	s			15.0	14.4–16.2
Extra-enriched (n = 28)	Fib-C***	g L^{-1}	1.53 (± 0.07)^{x,z}	4.8		
	TT	s	38.1 (± 3.58)	9.4		
	APTT	s			20.0	17.6–51.9
	PT	s			14.7	13.3–16.0

*** Means differ by $P < 0.001$ by ANOVA.

^{z} Means differ by $P < 0.001$ by ANOVA; ^{x} Means differ by $P < 0.01$ by ANOVA.

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Table 6 The impact of three different housing environments on the mean (± SD), median and range of clinical pathology parameters of rats.

Environment	Parameter	Unit	Mean (± SD)	CV%	Median	Range
Non-enriched (n = 25)	White blood cells†	10^9 L^{-1}	6.29 (± 1.47)^{zq}	23.5		
	Red blood cells*	10^{12} L^{-1}	8.43 (± 0.17)^{c}	2.01		
	Haemoglobin	mmol L^{-1}	9.13 (± 0.26)	2.87		
	Haematocrit	%	42.6 (± 1.4)	3.25		
	MCV	10^9 L^{-1}	50.54 (± 1.6)	3.21		
	MCH	fl	1.08 (± 0.03	2.93		
	MCHC	fmol	21.42 (± 0.55)	2.56		
	Platelets	10^9 L^{-1}	1,034 (± 107.6)	10.43		
	Reticulocytes	10^9 L^{-1}			175.0	150.0–236.9
	Neutrophils†	10^9 L^{-1}			0.54^{zx}	0.32–1.00
	Lymphocytes	10^9 L^{-1}	5.45 (± 1.34)	24.65		
	Monocytes*	10^8 L^{-1}			1.00	0.50–2.10^{c}
	Eosinophils	10^8 L^{-1}			0.90	0.60–2.00
	Basophils	10^8 L^{-1}			0.2	0.0–0.7
	Large unstained cells†	10^8 L^{-1}			0.3^{z}	0.1–1.3
Standard-enriched (n = 26)	White blood cells†	10^9 L^{-1}	5.37 (± 1.17)^{z}	21.8		
	Red blood cells*	10^{12} L^{-1}	8.42 (± 0.29)^{c}	3.41		
	Haemoglobin	mmol L^{-1}	9.19 (± 0.25)	2.77		
	Haematocrit	%	42.7 (± 1.4)	3.33		
	MCV	10^9 L^{-1}	50.70 (± 1.9)	3.72		
	MCH	fl	1.09 (± 0.04)	3.58		
	MCHC	fmol	21.57 (± 0.47)	2.19		
	Platelets	10^9 L^{-1}	986.9 (± 89.6)	9.07		
	Reticulocytes	10^9 L^{-1}			176.8	139.5–363.0
	Neutrophils†	10^9 L^{-1}			0.47^{z}	0.24–0.99
	Lymphocytes	10^9 L^{-1}	4.67 (± 1.17)	24.94		
	Monocytes*	10^8 L^{-1}			0.85	0.40–1.40^{c}
	Eosinophils	10^8 L^{-1}			0.90	0.50–1.40
	Basophils	10^8 L^{-1}			0.1	0.0–2.0
	Large unstained cells	10^8 L^{-1}			0.3	0.1–0.8
Extra-enriched (n = 28)	White blood cells†	10^9 L^{-1}	5.30 (± 1.77)^{q}	33.4		
	Red blood cells*	10^{12} L^{-1}	8.49 (± 0.24)	2.82		
	Haemoglobin	mmol L^{-1}	9.18 (± 0.27)	2.96		
	Haematocrit	%	42.8 (± 1.6)	3.68		
	MCV	10^9 L^{-1}	50.36 (± 2.07)	4.11		
	MCH	fl	1.08 (± 0.04)	3.26		
	MCHC	fmol	21.48 (± 0.53)	2.49		
	Platelets	10^9 L^{-1}	975.2 (± 96.0)	9.85		
	Reticulocytes	10^9 L^{-1}			183.7	132.7–244.0
	Neutrophils†	10^9 L^{-1}			0.38^{x}	0.22–1.15
	Lymphocytes	10^9 L^{-1}	4.61 (± 1.61)	34.98		
	Monocytes*	10^8 L^{-1}			1.00	0.40–2.00
	Eosinophils	10^8 L^{-1}			0.80	0.30–1.70
	Basophils	10^8 L^{-1}			0.2	0.0–1.4
	Large unstained cells†	10^8 L^{-1}			0.2^{z}	0.1–1.0

† Means/medians differ by $P < 0.05$ by ANOVA or Kruskal-Wallis test.
* Variances differ by $P < 0.05$ in Bartlett's or Levene's tests subsequently tested individually by *F*-test.
^{c,z,q} Means/medians differ by $P < 0.05$; ^{x} Means/medians differ by $P < 0.01$.

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Table 7 The smallest difference in normal distributed parameters, which is based upon the coefficient of variance found for three different environments that could be shown to be significant if $P < 0.05$ is set as cut-off, 25 animals are used and a power of 90% is demanded.

Parameter	Non-enriched	Standard-enriched	Extra-enriched
Cholesterol	17.5	12.4	16.4
Albumin	3.6	3.6	3.4
Protein	4.5	5.5	3.7
Phosphorus	12.4	13.9	12.0
Urea	9.7	9.3	9.0
Fib-C	6.1	4.0	4.5
TT	7.9	5.4	8.8
WBC	22.0	20.4	31.3
RBC	1.9	3.2	2.6
Haemoglobin	2.7	2.6	2.8
Haematocrit	3.0	3.1	3.4
MCV	2.2	2.5	2.8
MCH	2.7	2.4	2.2
MCHC	1.7	1.5	1.7
Platelets	7.1	6.1	6.7
Lymphocytes	16.7	16.9	23.6

Discussion

Overall, although we included 41 different parameters in this study, hardly any differences were observed between rats from the three different housing environments. Clearly, it is impossible to conclude that there will never be any differences in relation to these differences in housing conditions, but this study has increased our knowledge regarding the influence of environmental enrichment on biological parameters as has been requested (Baumans 2005; Hutchinson *et al* 2005; Weed & Raber 2005), and in terms of clinical pathology, haematological or cardiovascular pathology there is no basis, thus far, for denying rats environmental enrichment due the fear of a changed parameter expression or increased uncontrollable variation (van de Weerd *et al* 2004). We see no evidence within this field for environmental enrichment jeopardising the standardisation of experiments by increasing uncontrollable variation, and there is no reason to believe that a higher group size would be needed to study these parameters as has been discussed for behavioural parameters (Augustsson *et al* 2003; Tsai *et al* 2003; Sørensen *et al* 2010). If environmental enrichment has any effect on variation at all within this field, it is almost certainly too small to affect group size as pointed out previously (Eskola *et al* 1999; Augustsson *et al* 2003), as the group sizes in our study compare very favourably with the group sizes routinely used in research and regulatory testing, eg in clinical chemistry, haematology, telemetry and bleeding studies.

We acknowledge that environmental enrichment has a considerable effect on brain morphology, structure and biochemistry (Jones & Greenough 1996; Schrijver *et al* 2004; Bayne 2005), and in the absence of new models and research methods such changes may be detrimental to the research (Benefiel *et al* 2005). However, an impact observed within neurobiological research should not be automatically assumed to be something able to be extrapolated to other research fields. Also, it should be noted that in the present study as well as previously, we found little effect on behavioural and neurobiological tests when we compared the three different yet still standardised housing environments (Krohn *et al* 2010; Sørensen *et al* 2010). The difference between our studies and the experiences others may have had with enrichment may be that we used a fully standardised and commercialised set-up (as also recommended for comparing behavioural studies [Lewejohann *et al* 2006]) while enrichment, during its formative years, was typically unstandardised and lacked scientific merit.

We know from other studies that the rats themselves prefer an enriched environment (Krohn *et al* 2010) and, in relation to the claim made by Wolfer (2004) that the housing conditions for mice can be markedly improved without affecting the standardisation of results (Wolfer *et al* 2004), our studies would indicate that the same can be said for rats. On the other hand, it is also worth noting that rats do not appear to care whether the environment is enriched further (Krohn *et al* 2010) than the criteria stipulated by the Council of Europe (Stauffacher *et al* 2002), at least not in so far as it was in this study.

It could be claimed that had there been smaller variation in our study we might have been able to show a difference and, statistically, this is of course correct. However, the variation we observed was actually relatively low, despite the fact that we used an outbred stock of rats. Comparing the haematological data from our outbred Sprague-Dawley rats with the Taconic data sheets for their outbred Sprague-Dawley rats as well as their inbred Lewis rats (www.taconic.com) showed that of the nine data sets normally distributed in our study, we had smaller variation for all nine parameters, when compared with the Sprague-Dawley rat data sheet, and for seven out of nine parameters compared with the Lewis rats' data sheet. A power analysis ($\mu = 0.9$; $\alpha = 0.05$) of our data, for those normally distributed, shows that the difference we would have been able to show is considerably smaller than the difference normally expected in research.

Animal welfare implications

An enriched environment is preferred by rats (Krohn *et al* 2010), but during the last couple of years there have been concerns that it may jeopardise research (Jones & Greenough 1996; Tsai *et al* 2003; Schrijver *et al* 2004; van de Weerd *et al* 2004; Baumans 2005; Bayne 2005; Benefiel *et al* 2005; Hutchinson *et al* 2005; Weed & Raber 2005), which could lead some to accept that rats are denied enrichment. Based on the present study in male, Sprague-Dawley rats, we find no reason not to house rats under environmentally enriched conditions, as it has no influence on physiological and cardiovascular parameters or the variation between the animals. We would hope this finding will help consolidate the acceptance of such improved housing conditions for rats.

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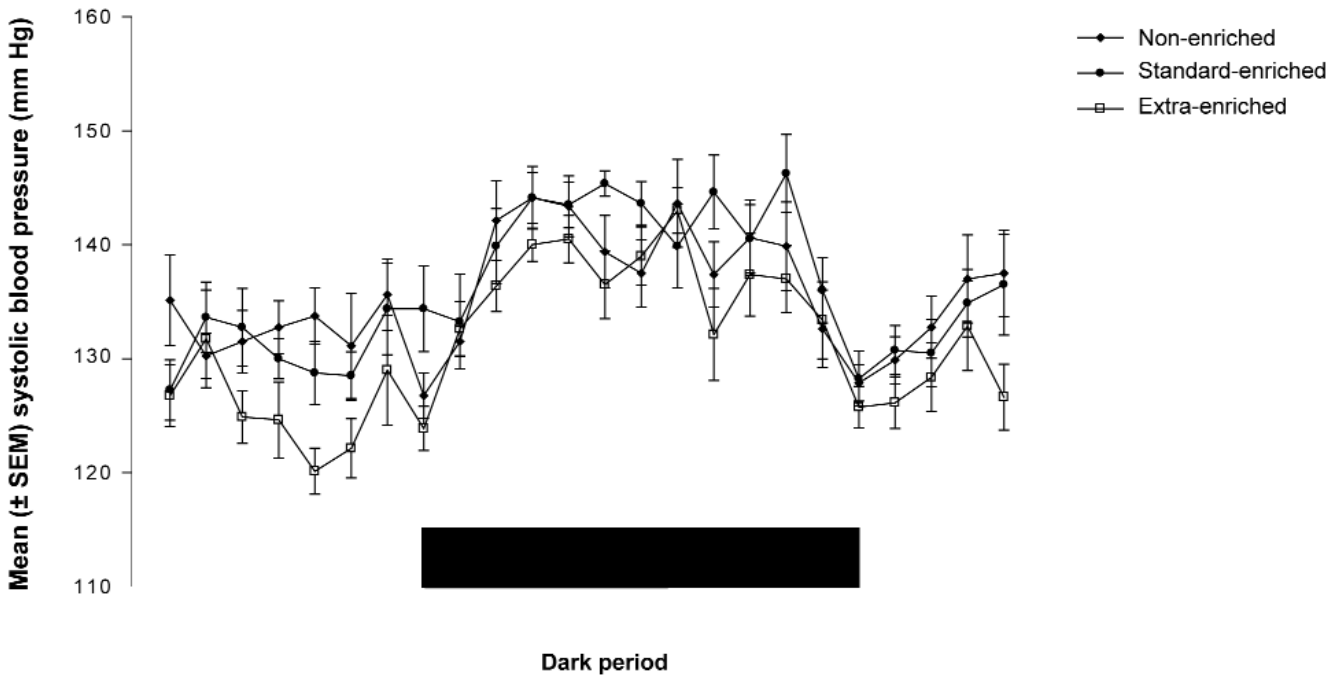
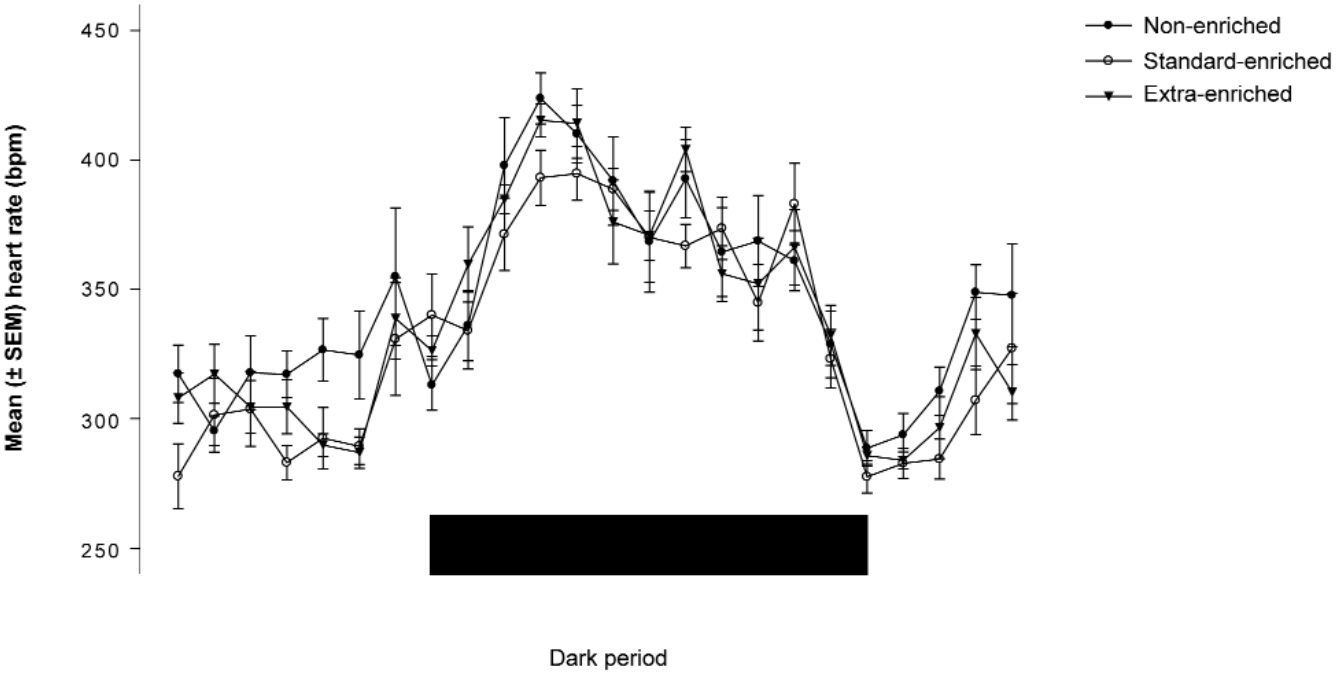
Figure 5



The impact of three different housing environments on mean (± SEM) systolic blood pressure in rats (n = 8) during a 24-h period. There are no significant differences between the housing environments.

Figure 6



The impact of three different housing environments on mean (± SEM) heart rate in rats (n = 8) during a 24-h period. There are no significant differences between the housing environments.

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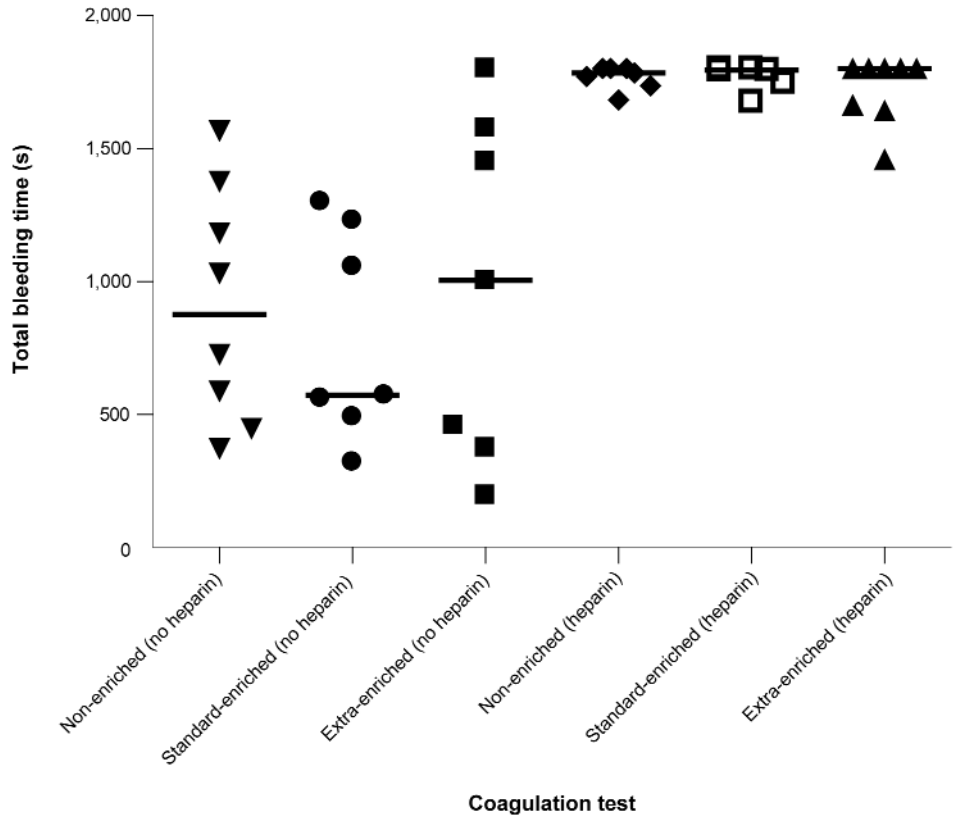
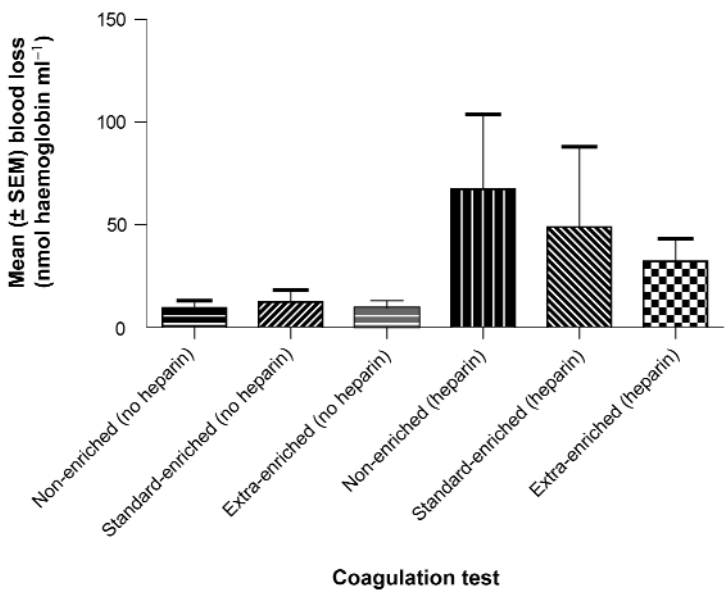
Figure 7



The impact of three different housing environments on the total tail bleeding time during a coagulation test either with or without heparin in rats (n = 6–8). There are no significant differences between the housing environments.

Figure 8



The impact of three different housing environments on the total mean (± SEM) blood loss during a coagulation test either with or without heparin in rats (n = 6–8). There are no significant differences between the housing environments.

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Conclusion

The Council of Europe working group for housing of rodents and rabbits (Hansen *et al* 2002) stated themselves that it is difficult to make a precise scientific documentation for very exact demands on housing. However, it is our conclusion that the minimum standards proposed and decided upon by the Council of Europe (being similar to the standard-enriched housing group [Figure 2]) would appear to constitute a reasonable compromise from the point of view that it has, at least, had little or no impact upon the type of research that we have studied.

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