The Influence of Enriched Environments on Learning and Memory Abilities in Group-Housed SD Rats

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Summary

Six traditional behavioural tests were done to evaluate the impact of inanimate enrichment on group housed animals. The study was performed in three parts to prevent the animals' previous experience influencing the behavioural tests. Three traditional housing environments with increasing levels of standardized inanimate enrichment were evaluated with regard to the effect on activity, fear, learning and memory abilities in socially housed rats.

The basic activity level of the animals was assessed using the open-field test. This test was combined with an amphetamine challenge test. The level of anxiety was evaluated by use of the elevated plus maze test. Secondly, a Morris water maze study was done to assess spatial learning abilities. Thirdly, two more complex learning ability tests were performed, namely the water Y-maze and the conditioned avoidance task.

The different housing conditions did not influence the level of activity, the level of anxiety or the response to amphetamine. Neither did the differences in housing conditions influence the learning abilities of the animals in the Morris water maze or the Y-maze. However, in the conditioned avoidance task, rats housed in the extra-enriched environment demonstrated significantly fewer avoidances than rats housed under non-enriched conditions.

Introduction

Evaluating the influence of an enriched environment, i.e. complex and stimulating housing conditions, on rats' mental abilities is important both to ensure the best possible welfare for the animals and to provide biomedical scientists with experimental animals that are behaviourally normal and physiologically healthy *(Hockly et al., 2002; Poole, 1997; Sherwin, 2004; Wurbel, 2001*).

From a welfare point of view, group housing is indeed of importance. Rats prefer to be housed with other rats and they also show a higher preference for social contact than for different kinds of physical enrichment items (*Patterson-Kane et al., 2002; Patterson-Kane, 2004*). Moreover, isolated rats have been shown to perform more tail-chasing and more escape-related behaviour than group-housed animals (*Hurst et al., 1997; Hurst et al., 1998; Sorensen et al., 2004)*.

Another point of concern is the validity of scientific data. To ensure optimal validity and reproducibility of research results, it is important to acknowledge the influence of the housing condition of experimental animals on the outcome of a variety of tests (see for

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example *Bowling et al., 1993; Hockly et al., 2002; Schrijver et al., 2004; Zhu et al., 2004)*. Studies have demonstrated that enrichment may influence both the mean value of the variables measured and the variability between groups. However, these results are not unambiguous, and more research is required *(Augustsson et al., 2003; Eskola & Kaliste-Korhonen, 1998; Spangenberg et al., 2005; Tsai et al., 2002; Krohn et al; 2011 ; Mikkelsen et al; 2010*).

One explanation for the apparent contradictory results may stem from differences in the tested environments. Environmental enrichment can be defined as "a combination of complex inanimate and social stimulation" (*Rosenzweig et al., 1978; van Praag et al., 2000*). This definition implies that physical complexities such as tunnels, nest boxes, nest materials and activity items as well as co-housing with conspecifics may be of importance. There is growing evidence that it is important to discern between these two basic ways of modulating the environment, namely by adding/removing physical stimuli/objects to modulate spatial complexity and by adding/removing social contact with conspecifics to modulate the social environment (*Schrijver et al., 2002; Schrijver et al., 2004; Varty et al., 2000; Wurbel, 2001; Zimmermann et al., 2001*).

Several studies have investigated the effect of the environment on the physiology and behaviour of rats. However, "enriched animals" are often both group-housed and provided with enrichment items beyond what could be considered normal procedures in a laboratory animal facility, whereas the "control groups" consist of single-housed animals in a barren environment (e.g. (*Spangenberg et al., 2005*). Thus it is not possible to discern between changes due to the physical environment and changes due to the social environment.

Stimulating housing conditions have proven to be important for the welfare of animals and the validity of data, but still it remains to be decided to what degree we can enrich the animals' environment without exposing the animal care staff to ergonomic hazards (Committee on occupational safety and health in research animal facilities *et al*., 1997) and without compromising the ability to compare present data to historical data obtained in previously used more or less non-enriched cage systems. In the present study, emphasis was placed on the inanimate environment of group-housed rats. A variety of traditional behavioural tests were applied to animals in three different standardized environments; the overall hypothesis being that as long as the rats are group housed (i.e. socially enriched/stimulated) there will be no effect on the outcome in these tests of inanimate enrichment traditionally used for laboratory rats. Therefore, three standardized environments with increasing level of inanimate enrichment/complexity were evaluated with regard to the effect on activity, fear and learning and memory abilities in rats housed in groups of four.

Research has shown that a complex and stimulating environment induces changes in neurochemistry and neuroanatomy mainly in the cortex and hippocampus. The performance in spatial learning and memory tasks depending on hippocampal and neocortical functions is better in rats from enriched environments compared to rats housed in barren environments that have retarded abilities (see for example *(Bernstein, 1973; Farrell et al., 2001; Rosenzweig & Bennett, 1996; Schrijver et al., 2004; van Praag et al., 2000*)). This effect, when assessed in the Morris water maze, has been shown to be independent of the social environment (*Schrijver et al., 2002*). In this study, we evaluate the influence on the hippocampal and neocortical functions by use of the Morris water maze, a Y-water maze and conditioned avoidance. Social isolation, on the other hand, mainly affects corticostriatal dopaminergic pathways and enhances dopaminergic activity in the nucleus accumbens resulting in hyperactivity and increased exploration (*Fulford & Marsden, 1998; Hall et al., 1998; Hall, 1998; Heidbreder et al., 2000; Schrijver et al., 2002; Winterfeld et al., 1998*). The animals in this study were not isolated, and hence we did not expect any effect on activity and the behavioural response to the amphetamine challenge test.

To sum up: in this study, six traditional behavioural tests were done to evaluate the impact of inanimate enrichment on group housed animals. The study was performed in three parts to prevent the animals' previous experience influencing the behavioural tests. First, the level of anxiety was evaluated by use of the elevated plus maze test. The basic activity level was evaluated using the open-field test and the animal's response to amphetamine injections was tested.
The second phase consisted of a Morris water maze study. For the third phase, two more complex learning ability tests were done, namely the water Y-maze and the conditioned avoidance task.

Material and Methods

Animals & housing

The housing of animals was identical throughout the study. A total of 156 outbred Spraque-Dawley (NTac:SD) male rats were obtained from Taconic Europe, Denmark, for all three phases of the study. The rats were all four weeks old at arrival and they were housed in the assigned environment for a minimum of 12 weeks prior to testing. For evaluation of the elevated plus maze, open field and amphetamine challenge test, 60 animals were used; 36 were used for the Morris water maze and 60 animals were used for the water Y-maze and the conditioned avoidance task.

All animals were marked by ear notching on arrival. The animals were randomly assigned to one of three housing conditions with increasing level of enrichment, and a group size of four animals per cage:

1) Non-enriched cages (NE). A standard type IV cage (Tecniplast, Buguggiate, Italy), 18 cm high with aspen bedding only (Tapvei, Kortteinen, Finland).
2) Standard-enriched cages (SE). A standard type IV cage, 18 cm high with 700 grams aspen bedding, 100 grams Enviro-Dri® nesting material (Lillico Biotechnology, UK), aspen brick (size M; Tapvei, Kortteinen, Finland) and a 13 x 15 x 20 cm, black-transparent plexiglas rat shelter (Repsol, Brønderslev, Denmark).
3) Extra-enriched cage (EE). A Scantainer Novo type IV cage with a raised lid, 32.5 cm high with a built-in platform for resting and lookout (Scanbur, Karlslunde, Denmark). The cage was supplied with 700 grams aspen bedding, 100 grams Enviro-Dri® nesting material, aspen brick and a rat shelter.

All three environments can be considered standardized as the cages and the enrichment items are all commercially available.

The cages were allocated in a systematic and balanced manner to a Scantainer Novo rack (Scanbur, Karlslunde, Denmark). Each cage had its own permanent position in the rack, and the three different environments were mixed to avoid bias due to different locations in the Scantainer Novo rack (e.g. on high and low shelves). The animals had free access to Altromin 1324 chow diet (Brogaarden/ Altromin GmbH) and tap water. Fresh water was provided twice a week. The cages were cleaned twice a week. Enrichment items such as shelters and bricks, but not nest material, were either transferred to the clean cage or replaced when necessary.
All animals were housed in the same room, and in both the animal room and the experimental rooms the temperature was 20 ± 2 °C and the relative humidity 40–60%. Artificial lighting was on from 06.00 to 18.00 h. There was no daylight in the animal rooms.

Test procedures

Elevated plus maze

The animals were tested in the elevated plus maze at 17 weeks of age. The elevated plus maze consisted of two open arms perpendicular to two closed arms (each arm was 12 cm wide and 50 cm long) extending from an open central area (12x12 cm). All parts of the apparatus were constructed of light grey painted plywood. The plus maze was placed in a test room, shielded with screens, and the animals were observed via a closed-circuit video camera mounted in the ceiling.

Testing was conducted during the light part of the light–dark cycle. The animal was placed in the central area, facing the open arm away from the handler, and the behaviour of the animal in the maze was videotaped. The duration of the test was 10 minutes. The maze was thoroughly cleaned with 70% ethanol after each successive test.

Behavioural analysis was conducted using the computerized tracking and analysis system Ethovision (Noldus Information Technology, The Netherlands). For the elevated plus maze, distance moved, number of visits and total time spent were evaluated in each of the three compartments (the open arms, the closed arms and the centre of the maze). The video tracks of the animals were analysed in Ethovision and transferred to the Statistical Analysis System SAS, which was used for all statistical analyses throughout the study (SAS Institute Inc., version 9.1) with a 5% significance level. Data that were normally distributed were analysed using an analysis of variance (ANOVA) on the outcome variables (distance moved in the three zones, frequency of visits and time spent in each of the three zones). In addition, Bartlett's test for equal variances in the three environmental groups was performed.

Open-field and amphetamine challenge tests

The amphetamine challenge study, including the open-field test, was done when the animals were 19-21 weeks of age. The test arena was circular measuring 100 cm in diameter and 40 cm in height. Three arenas were used allowing simultaneous testing of three animals (arena 1, 2 and 3). The arenas were constructed of thin metal sheets painted light grey. The arena was well lit (470 lux in the centre of the open field, 10 cm above the floor of the arena). The arenas were shielded with screens and the animals were observed via a closed-circuit video camera mounted in the ceiling.

The rat was placed in the centre of the field and allowed to habituate to the arena for 30 minutes before dosing with d-amphetamine. The first 10 minutes of this habituation period was equivalent to the open-field test. The activity of the animals was measured throughout the period to allow an assessment and differences in habituation between the groups. After 30 minutes the animals were injected with d-amphetamine, immediately returned to the open field and observed for another two hours. Three doses were used for constructing the dose-response curve: 0.25 mg/kg, 0.50 mg/kg and 1.00 mg/kg. From each of the three housing environments, 6-8 animals were used for every dose level (Table 1). Each animal was only dosed and tested once. Due to the number of test animals, the total test period was 10 days (resulting in a statistically independent variable "day"). Half of the animals were tested in the morning and the other half in the afternoon (independent variable "time"). The arenas were cleaned with a mild detergent between tests. Total time in the field was 2.5 hours.

Open-field data were obtained using the data from the first 10 minutes in the field. A dose-response curve was constructed for the entire period with the first 30 minutes being the habituation period, and the last two hours being the amphetamine challenge response.

In the Amphetamine challenge test, data were collected for periods of 5 minutes for 2.5 hours with a total of 30 measures per animal.

Table 1. Amphetamine challenge test: Dosing and number of animals in the three different environments. NE= Non-enriched; SE = standard enriched; EE = Extra-enriched.

Dosing	Environment			Total
	NE	SE	EE	
0.25 mg/kg	8	7	7	22
0.50 mg/kg	6	7	6	19
1.00 mg/kg	6	6	7	19
Total	20	20	20	

The Morris water maze

The rats were 18 weeks of age at test start. The animals are divided into two groups; 1 and 2. Two rats from each cage were trained and tested the first week (group 1), and one week later the remaining rats (group 2) were trained and tested.

The water maze consisted of a black, circular tank, measuring 150 cm in diameter and 50 cm in height (Bonar Plastics/Metas, Smørum, Denmark). The tank was filled with tap water to a level 20 cm below the rim. The water was changed twice a week and maintained at 21 ± 1ºC. A transparent, square platform (11 cm) with a rough surface to facilitate escaping the water was placed in either of four virtual quadrants (NE, SE, SW, NW), 30 cm from the wall. The platform was submerged 2 cm below water level during spatial navigation. For probe trials, the platform was removed. The tank was placed in a test room, shielded with screens marked with a variety of distinct external maze visual cues. Eight equally spaced points on the wall of the tank were designated as N, NE, E, SE, S, SW, W and NW and were used as release points. Swim paths were recorded by a video camera mounted above the centre of the pool.

Prior to testing, all rats were habituated to a type III macrolon cage with aspen bedding used for individual transportation. To assess spatial navigation learning, the animals were first trained on 5 consecutive days with 4 daily trials to locate the hidden platform. The platform was placed in a fixed position during these trials. For half of the rats of each treatment group (n = 6), the platform was placed in position S; for the other half (n = 6) in position N. On Days 4, 5 and 6, a probe trial with no platform was performed prior to normal training to assess memory formation for the trained platform position.

Training trials lasted for 90 s. The rat was released at the edge of the pool, facing the wall, in a randomised position (N, NE, E, SE, S, SW, W, NW) at each successive trial. Rats that did not find the platform within 90 seconds were guided to it by the experimenter. After 30 seconds on the platform rats were removed from the tank, followed by a 30 second intertrial period.

The probe trials lasted for 60 seconds. In probe trials, rats were always released opposite the trained platform position.

During the intertrial periods the rats were placed in their individual transport cages, which were permanently placed on a heating pad during the daily sessions. Following the last trial of a daily session the rats were gently rubbed dry with a towel.

The video tracks were analysed using Ethovision. The swim paths of the probe trials on Days 4 and 6 were analysed for length of swim path distance travelled, cm, swim speed (cm/s), latency to reach the platform area (seconds) and time spent searching in each of the four quadrants (seconds). If a rat failed to search the platform area, the latency time was set to 60 seconds.

Normally distributed data were analysed using an analysis of variance and Bartlett's test was done to compare the variance within the three housing conditions. Data, that were not normally distributed, were analysed using an ANOVA on ranked data.

Y-water maze

A total of 29 animals were used for this study. A total of 60 animals were housed in groups of four in one of the three test environments (20 animals in each environment). Two animals were randomly chosen from each cage. One animal died prior to testing; hence only one animal was used from that cage. The animals were tested at 17 weeks of age.

The Y-water maze consisted of a black, circular tank measuring 140 cm in diameter and 50 cm in height. The tank was filled with tap water to a level 15 cm below the rim. Inside the pool, metal walls formed a Y-maze. The arms were 45 cm long and 15 cm wide. A removable, transparent plexiglas platform was positioned at the extremity of one arm with the top of the platform 0.5 cm below the surface of the water. The tank was placed in a test room, shielded with screens marked with a variety of distinct external maze visual cues.

The animals were habituated to the test room for

two hours prior to test start. At test start the test animal was carefully placed in the southern maze arm (the "drop arm"). The test animal was allowed to explore the maze, and latency to find the hidden platform was noted. Upon finding the platform the animal was allowed 30 seconds on the platform to orientate itself. If the animal's search time exceeded 90 seconds the animal was placed on the platform by the experimenter. After an intertrial interval of 10 seconds the animal was reintroduced into the drop arm of the maze. Moreover, the number of wrong arm entries was noted. An arm was considered visited when the base of the animal's tail was in the arm. These trials, in which the position of the maze is learned without prior experience of the maze, were called start trials. When an animal had had 5 consecutive correct responses (no wrong arm entries), the platform was moved to the opposite arm ("reversal trials"). Again, the task was considered learned when the animal showed five consecutive correct responses. All observations including latency time and errors were noted by the experimenter.

The parameters used for statistical analysis of the Y-maze reversal learning were "total time spent" in start trials and reversal trials and "total number of errors" in start trials and reversal trials. One animal from the extra-enriched environment was removed from the data as after three trials he started to avoid the arm with the platform and instead successfully and repeatedly escaped the maze by jumping directly from the water.. The parameters used for analysis of the conditioned avoidance task were number of avoidances, number of escapes and number of failures to escape.

As none of the data were normally distributed, an ANOVA was performed on ranked data.

Conditioned avoidance

A total of 59 animals were used for this study, as one animal of the original 60 died prior to testing. Testing was done when the animals were 18 weeks of age.

Conditioned avoidance testing was conducted using four automated two-way shuttle boxes (ENV-010M; MED-Associates, St. Albans Vermont, USA) each placed in a sound attenuated chamber. The boxes were subdivided into two compartments by a partition with an opening. The position of the animal and crossings from one compartment to the other were detected by two photocells placed on either side of the dividing wall. Upon presentation of the conditioned stimuli (CS), tone and light, the animals had 10 seconds to cross to the other compartment of the shuttle box in order to turn it off and thus end the trial avoiding the appearance of the unconditioned stimulus (UCS). If the animal remained in the same compartment for more than 10 seconds, the UCS was presented as 0.5 mA scrambled foot shocks until escape was made or for a maximal duration of 10 seconds. To evaluate the learning ability of the animals the following behavioural variables were evaluated for each training session: number of avoidances (response to CS within 10 seconds, i.e. moving to the other compartment when the tone was heard, and the light was turned on), number of escapes (response to CS + UCS, i.e. the animal moved to the other compartment when it received the foot shock), number of escape failures (failure to respond, i.e. the animal stayed in the compartment, accepting the foot shock).

The rats were habituated to the shuttle box 3 minutes before each session. Training was carried out on 3 consecutive days. Each training session consisted of 10 trials with intertrial intervals varying randomly between 20 and 30 seconds. There were a total of five training sessions per day.

Three behavioural responses were analysed, namely active avoidance, escape and failure to escape.

Results

Elevated plus maze

Data were normally distributed. The level of activity in the elevated plus maze (total distance moved), number of visits in the three compartments and time spent in each of the three compartments did not differ between the animals from the three different environments. No significant difference was found when comparing the variation within the three environments.

Animals from all three environments spent significantly more time in the closed arms compared to the open arms of the maze and the centre (non-enriched (NE); standard-enriched (SE); extra-enriched (EE): $p<0.0001$). Also, in all environments, the animals spent significantly more time in the centre compared to the open arms (NE: $p= 0.0015$; SE: $P = 0.0250$; EE: $p<0.0001$).

Open field

Both the outcome variables (total distance moved) in the entire arena and time spent in the two zones (centre and periphery) were not normally distributed and hence an ANOVA on ranked data was performed

No differences were found between the three environments in activity (total distance moved in cm) and in time spent in the centre and in the periphery of the open field. However, a significant effect of day was found ($p= 0.0088$) with the lowest levels of activity found on the first days of the test (Table 2).

Table 2. The open field test. Total distance moved (cm +/- std dev.). A significant effect of day was found ($p= 0.0088$) with the highest level of activity seen later in the test period.

	N	Open field Arena, total distance moved
Day 1	6	1900.37 ± 809.02
Day 2	6	2362.31 ± 417.94
Day 3	6	2363.64 ± 679.15
Day 4	6	3220.65 ± 551.71
Day 5	6	3203.26 ± 698.91
Day 6	6	2864.39 ± 673.14
Day 7	6	3104.89 ± 885.90
Day 8	6	2899.92 ± 910.91
Day 9	6	3064.93 ± 628.84
Day 10	6	3573.59 ± 953.63
Total	60	

Amphetamine challenge

Two animals (one from the non-enriched environment and one from the extra-enriched environment) were excluded from the statistical analysis due to technical problems with tracking of the animals. The following independent variables were included in the initial model: environment, time of day and arena used (1, 2 or 3). Moreover, a dose of amphetamine was included as an independent variable in the dose-response period. Cage number and day were included as random effects.

As data from the habituation period were normally distributed, the outcome variable "distance moved" was analyzed using a repeated measures ANOVA. Due to non-normality, data for the dose-response period were square root transformed prior to a repeated measures analysis of variance. Finally, the "total distance moved" in the two periods (habituation and dose-response period) and the "maximum level of activity" (longest distance moved during a 5-minute interval) were analyzed using an analysis of variance with cage as a random effect. Both variables were normally distributed.

During habituation, which was 30 minutes prior to dosing, the activity in each of the six five-minute intervals was neither affected by housing environment, arena, cage number, time of day nor test day. However, the time intervals (1-6) significantly influenced the distance moved (Table 3), with the highest level of activity found in the first intervals

Table 3. Habituation and activity. Mean distance moved (cm) in the 6 five-minutes intervals of the habituation period. Means with different letters are significantly different ($P< 0.01$).

Interval	Mean distance moved	Std. error	
1	1397.88	64.66	a
2	1452.66	64.66	a
3	1247.47	64.66	b
4	1040.37	64.66	c
5	885.39	64.66	d
6	802.44	64.66	d

and significantly lower levels at the last intervals. During the dose-response period only an effect of interval was found ($p<0.0001$), the distance moved in a five-minute interval being significantly higher in the beginning of the two-hour period. No effect of dose, environment, time of day, the arena used or day was found for the maximum distance moved in the two periods. For the total distance moved, no significant effects were found on the dose response curve; however, for the habituation period, an effect of day was found ($p = 0.0262$).

Morris water maze

The data on the total distance travelled, mean swim speed and latency to reach the platform area were normally distributed and analysed using the ANOVA. No differences between the three environments were found (Table 4), and no effects were found on either cage, probe trial number (Day 4 or Day 6) or week of testing. There was no significant difference in the variance in the three environments.

Comparing the four quadrants during the probe trials demonstrated that the time spent searching in each quadrant was significantly influenced by the position of the platform during training trials (data not shown). There were no effects of housing conditions, cage, probe trial number or week of testing, and all rats - regardless of housing conditions - searched significantly more in the area where they expected the platform to be.

Y-maze

No differences were found between the three housing environments with regard to total number of errors or total time spent for searching for the platform in start and reversal trials.

Conditioned avoidance

None of the measured parameters (active avoidance, escape and failure to escape) were normally distributed, and hence an ANOVA was applied to ranked data. The data were analysed for each of the three test days, comparing the three housing environments. As testing was done over three days, a repeated measures ANOVA was used. In case of significant effects, pair wise comparisons of significant variables were done using the least-squares means (LSM) procedure.

The number of avoidances (Table 5, Figure 1) varied significantly between non-enriched and extra-enriched housing environments on Day 2 and on Day 3. In both cases, rats housed in the non-enriched environment demonstrated significantly more avoidances than rats housed in the extra-enriched environment. Regarding number of escapes (Table 5), animals from the standard-enriched environment and the extra-enriched environment demonstrated significantly more escapes on Days 1 and 2 than non-enriched animals. On the first test day, animals from the non-enriched environment had significantly more escape failures (Table 5) than the other groups.

Discussion

In the present study, no effects of the different environments were found on standard tests such as open field, elevated plus maze and Y-maze. Nor was the performance in Morris water maze

Table 4. Path length (mean distance; cm. ± std.dev) and swim speed (cm/s ± std.dev) in the water maze during 60 seconds probe trials under different housing conditions.

Housing condition	N	Mean distance ± std.dev	Mean swim speed ± std.dev
Non-enriched	23	1425.81 ± 211.63	23.95 ± 3.6
Standard Enriched	24	1410.60 ± 180.47	23.62 ± 3.03
Extra-enriched	22	1483.20 ± 186.84	24.87 ± 3.12

Table 5. Total number of avoidances, escapes and escape failures (+/- SEM) in the conditioned Avoidance task. Significant statistical differences (LS means procedure) in the three parameters between different housing conditions are shown. NE= Non-enriched; SE = standard enriched; EE = Extra-enriched. Within each group, days with different capital letters indicate a significant difference between days.

	Total number of Avoidances			**Total number of Escapes**			**Total number of Escape failures**		
Day	NE	SE	EE	NE	SE	EE	NE	SE	EE
1	12.5 ± 2.4	6.9 ± 1.4	7.8 ± 2.2	26.0 ± 3.0	38.3 ± 2.7	36.3 ± 3.1	11.8 ± 3.5	4.85 ± 2.8	5.89 ± 2.7
				0.0041	**		0.0148	*	
				0.0174		*			
		A			A				
2	21.1 ± 3.9	12.5 ± 2.9	9.1 ± 2.5	20.6 ± 3.4	30.2 ± 3.4	31.2 ± 3.2	8.3 ± 3.4	7.7 ± 3.4	9.6 ± 3.3
	*0.0466		*	0.0485	*				
				0.0316		*			
		A			A				
3	24.4 ± 3.7	22.0 ± 3.5	13.0 ± 3.5	19.4 ± 3.0	19.6 ± 3.1	27.8 ±4.0	6.3 ± 3.0	8.4 ± 3.9	9.2 ± 3.9
	*0.0368		*						
		B			B				

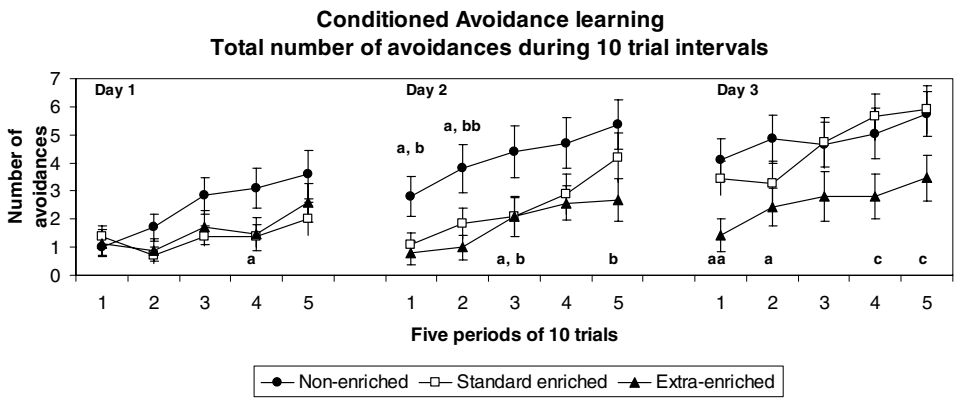

Figure 1. Total number of avoidances in the five test periods of 10 trials (a total of 50 trials per day). a = significant difference between non-enriched and enriched environment; a: 0.01 <p=< 0.05, aa: 0.001<p=<0.01. b= significant difference between non-enriched and ScantainerNovo; b: 0.01 <p=< 0.05, bb: 0.001<p=<0.01. c= significant difference between enriched and ScantainerNovo housing; c: 0.01 <p=< 0.05.

and amphetamine challenge test influenced by housing environments. It should be remembered that all animals were group-housed, and moreover, they did have bedding as a minimum. This setup could indicate that the importance of the social environment outranks the importance of differences in non-animate enrichment like shelter, biting bricks and nesting material.

Drugs such as amphetamine that stimulate dopamine release in the mesolimbic pathways will induce behavioural changes such as hyperactivity and, at larger doses, stereotyped sniffing and grooming (*Creese & Iversen, 1973; Giros et al., 1996; Sills et al., 1999*). In rats housed in an enriched environment, a larger increase in the locomotor activity following amphetamine challenge has been observed when compared to rats single-housed in an impoverished environment (*Bowling et al., 1993; Zhu et al., 2004)*. As all the animals in our studies were group-housed, we did not expect to find any differences.

The results of the open-field test show a significant influence of day on the activity. This result could indicate that the rats are disturbed by the handling of the cages and the testing, since this effect is most pronounced on the first three days of testing. After day three, rats from all cages have been tested, and hence all rats have been disturbed by the removal of cage mates etc., and may thus be more active in the open-field test. This effect of day is also seen in the habituation period in the amphetamine challenge test, which is not surprising as the open-field test (10 minutes) is actually the first third of the 30-minute habituation period. However, this effect of day disappears after dosing. It is likely that the increased level of activity masks any minor differences due to an effect of day.

In the Morris water maze no differences in learning ability were found. As none of these animals had received any treatment severely impairing their cognitive ability, this result is not surprising.

In the reversal learning test all the animals learned the task at the same speed. Moreover, moving the platform, requiring the animals to learn the new position, did not reveal any deficits in any of the groups, either. However, it is noteworthy that the experimenter observed that one of the extra-enriched animals apparently consciously avoided the arm with the platform after having been removed and put back in the pool a few times. Instead, the rat successfully jumped up the sides of the pool and escaped. This rat was removed from the data set , but it should be acknowledged that this animal learnt fast, even though it was not the task planned by the experimenter.

In the conditioned avoidance task the obtained results seem contra-intuitive as the extra-enriched animals demonstrate fewer numbers of escapes than the two other groups of rats on test Days 2 and 3. Or, in other words, they did not seem to learn the task properly. Moreover, even though they did improve in the course of a day, the next day the extra-enriched rats behaved as if they did not – to the same extent as the other groups - remember what they learned the day before (Figure 1). Both of the enriched groups had overall more escapes on the first days, i.e. they only moved when given the foot shock. On the other hand, the non-enriched animals did demonstrate significantly more escape failures on the first day compared to the enriched group.

As no differences in learning abilities were found in the Morris water maze, and the literature consistently ascribes better learning abilities to enriched animals, we have no reason to assume that enriched animals are poorer learners. Thus, other explanations for the findings in the conditioned avoidance task must be explored.

It is noteworthy that the non-enriched animals had significantly more escape failures than standard enriched on the first day, which of course may explain the lower number of escapes. Basically, it could be concluded that non-enriched rats on the first day take more foot shocks than the other groups, because they do not move to the other compartment of the test box. The total number of avoidances is the same for all three groups on the first day. Over the next two days the number of escape failures becomes the same in the three groups. The number

of escapes goes down and the number of avoidances goes up, though only significantly in the standard-enriched group (Table 5), but non-enriched animals do have significantly more avoidances than the extra-enriched animals. Moreover, the extra-enriched animals do not seem to improve their avoidance rate over the three days (Table 5), and neither do the non-enriched animals, whereas the standard-enriched significantly do. Table 5 seems to indicate that non-enriched animals quickly learn the task and so do the standard-enriched ones, even though they do not learn quite as fast. Extra-enriched animals demonstrate the same number of escape failures, but have significantly more escapes on the first days.

It could be hypothesized that the extra-enriched animals in some way are more indifferent to the pain related to the foot shocks, but this should have been reflected in more escape failures. If pain matters less, then why move? On the other hand, the foot shocks may be slightly annoying, making the animals move, even though they do not find it worthwhile leaving in advance. Such an effect of reduced pain perception could be related to a higher level of acute stress in the extra-enriched rats inducing an analgesic effect (*Pinto-Ribeiro et al., 2004; Vendruscolo et al., 2004*) due to a higher sensitivity to the aversiveness of the test chamber. On the other hand, one could speculate that these animals simply are more resistant to aversive stimuli due to better coping abilities induced by a more complex housing environment.

A study by Barbelivien *et al* (*2006*), however, demonstrated that rats housed in an enriched environment had an increased ability to process contextual information, and hence showed more contextual fear than non-enriched rats. Moreover, the enriched rats failed to demonstrate increased fear conditioning to an auditive stimulus (a sound cue), and they also showed reduced fear response when re-exposed to the sound cue compared to standard-housed rats (*Barbelivien et al., 2006*). A similar mechanism could help to explain why the extra-enriched rats in our study failed to condition to the sound cue.

Conclusion

The three standardized levels of enrichment did only influence the animals' performance in the conditioned avoidance task. The overall results of the study indicate that enriching the cages with a variety of items, still ensuring an easy to handle cage type, will not influence the results of an experiment and thus the ability for the researchers to compare their data to historical references obtained using cages with another level of enrichment.

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