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Psychological stress and environmental adaptation in enriched vs. impoverished housed rats

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Abstract

In this study, we report differential behavioural and cognitive effects, as assessed in the open-field and the Morris water maze, following psychological stress in enriched vs. impoverished housed rats. Three stress conditions were evaluated: nonstress, mild stress and powerful stress. Mild stress consisted of exposure to an avoidance box but without shock, while in the powerful stress condition animals were exposed to an electric shock. The results revealed distinct effects in the differentially housed animals. Prior exposure to a mild stress enhanced escape performance in the water maze in enriched but not impoverished animals. However, preexposure to powerful stress negatively affected animals from both housing conditions in the water maze task, but with the enriched animals less affected than impoverished animals. In the open-field test, stress preexposure reduced locomotion counts in both the differentially housed animals. In addition, the results showed that the enrichment effect on emotional reactivity in the open-field is long-lasting and persists even after extensive training and housing in standard laboratory conditions. The results are discussed in relation to the nature of the behavioural and learning differences between the differentially housed animals. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Stress; Learning; Memory; Open-field behaviour; Enriched; Impoverished; Environment; Corticosterone

1. Introduction

Many studies have documented the relationship of levels of corticosterone with cognitive function. Enhancement of corticosterone levels by restraint stress, which caused atrophy of apical dendrites in the hippocampal CA3 neurons, resulted in impaired performance of spatial learning (Luine et al., 1996). Furthermore, implants that allowed slow release of corticosterone over 3 weeks also caused spatial learning impairments in rats (Dachir et al., 1993) and resulted in specific damage to pyramidal hippocampal neurons mainly in CA1 and CA4 regions (Arbel et al., 1994). While long-term effects of corticosterone through sustained exposure to restraint stress or corticosterone pellets have deleterious effects on cognitive function, there is considerable evidence showing that short-term exposure to low levels of corticosterone can enhance cognitive function. While low levels of stress hormones elicited by mild stress can improve cognitive function, elevated levels as those induced by chronic stress impair cognitive function (Conrad et al., 1999; Diamond et al., 1992; Lindau et al., 2000; Lupien and McEwen, 1997; McEwen and Sapolsky, 1995; Sandi, 1998; Sandi and Rose, 1994a,b, 1997). Of interest are also observations linking hippocampal plasticity with corticosterone levels (Bennett et al., 1991; Diamond and Rose, 1994; McEwen and Sapolsky, 1995; Sapolsky, 1990). It has been shown that psychological stress, such as exposure to predators, and footshock can impair memory (Diamond et al., 1996; Jodar et al., 1995; Park et al., 2001). In contrast to the well-established learning and memory effects of corticosteroids, less is known about their behavioural effect per se. Psychopharmacological studies have shown that administration of low, but not high doses, of corticosterone decreases exploratory and investigatory behaviour in open-field and in the Morris water maze (Oitzl and de Kloet, 1992; Oitzl et al., 1994, 1997).

Studies comparing animals with different environmental history such as neonatally handled vs. nonhandled animals have shown acute as well as stress-related long-lasting differences (Meaney et al., 1988). The effects of neonatal handling and adult stimulation are observable at the physiological

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(Meaney et al., 1988; Diamond et al., 1992; Henriksson et al., 1992; Mohammed et al., 1993; Pham et al., 1999a) as well as at the psychological level (Renner and Rosenzweig, 1986; Mohammed et al., 1986, 1990; Rosenzweig, 1996; Pham et al., 1999b; Lindau et al., 2000).

It is well-established that housing rats in a stimulating/enriched environment (large cages with stimulus objects) compared to housing in a nonstimulating/impoverished environment (housing in isolation) induces a number of neurochemical, neuroanatomical and behavioural alterations (Bennett et al., 1964; Diamond, 2001; Greenough, 1975; Kempermann et al., 1997; Rosenzweig, 1996). Behaviourally animals from enriched environment perform better in learning tasks compared with animals from impoverished environment (Mohammed et al., 1986, 1990; Renner and Rosenzweig, 1986, 1987; Pham et al., 1999a) and they show faster emotional adaptation in novel situations (Mohammed et al., 1990; Falkenberg et al., 1992; Fernández-Teruel et al., 1997; Pham et al., 1999b).

Since animals from enriched environment show less behavioural stress in novel environments, one hypothesis that has been advanced to account for their cognitive enhancement is that they have a more adaptive HPA axis response system (Uphouse, 1980; Mohammed et al., 1993). However, no significant differences in basal corticosterone levels between enriched and impoverished animals have been reported (Pham et al., 1999b), nor in their corticosterone levels following stress (Larsson and Mohammed, unpublished observation). Furthermore, while some earlier work indicated a difference in adrenal weight between enriched and impoverished animals (Krech et al., 1966), subsequent studies failed to demonstrate any critical importance of corticosterone in the salutary effects of enriched environment (Devenport et al., 1992). However, we have previously reported that enriched housed animals have higher expression of GRs in hippocampus in comparison to impoverished housed animals (Mohammed et al., 1993; Olsson et al., 1994), and others have shown variations in relative MR/GR distribution in the brain of normally housed rats (McEwen et al., 1986).

Psychological stress is one of the most potent stressors for the organism and it triggers corticosterone secretion in an intensity-dependent manner. Fluctuations in corticosteroid levels can be said to reflect emotional states related to stress. Hennessy (1991) and Hennessy et al. (1979) demonstrated that different levels of corticosterone reflected different levels of stimulus intensity. Rats that were exposed to three increasingly unfamiliar environments showed three corresponding elevations in mean plasma corticosterone levels. Thus, rats that were exposed to a novel cage showed higher levels of stress hormones than those exposed to a cage that was not so novel (i.e., a cage that was similar to the home cage). In the present study, we sought to expose differentially housed animals to differing degrees of stress intensity levels and study the effects on behaviour. Animals were exposed to a mild stress condition or a powerful stress condition and later behaviourally tested. The mild stress condition consisted of placing animals in a passive avoidance box without shock exposure; while in the powerful stress condition, the animals were exposed to the box and shock. The shock exposure in a novel box was expected to cause an increased release of stress hormones, in comparison with the mere exposure of animals to the box without shock.

In a recent study, we examined the behavioural effects of stress-dependent variations in corticosterone secretion. We hypothesised that different behavioural and cognitive effects would appear after exposure to psychological stress and that these effects would vary depending on stress intensity (Larsson et al., submitted for publication). We adapted a method for evaluating these cognitive and behavioural effects during the HPA axis predominated phase. The animals were exposed to a short bout of psychological stress (90 s) and were then left undisturbed until testing started 15 min later. Three conditions were employed in which two served as stressors (nonstress, mild stress and powerful stress). All treatments were evaluated for acute (15 min) and long-lasting (15 days) effects. The main results from that study showed that psychological stress prior to test increased exploratory behaviour without significantly affecting cognitive abilities. An unexpected result appeared also, which showed that one exposure to an aversive treatment (shock) could have rather long-lasting behavioural and cognitive effects. The present findings extend these results.

In the present study, we aimed to do the following.

(1) Replicate and further explore our earlier findings of behavioural and cognitive differences in differentially housed animals. The new parameters measured were long-lasting effects of enrichment on emotional reactivity (open-field), and enrichment induced differences in search behaviour in the Morris water maze task. The hypothesis was that the enriched animals will habituate faster in the open-field test, and that they would learn and remember the Morris water maze better than the impoverished animals. In view of their earlier stimulating experience, the enriched animals were expected to show behaviourally less long-lasting effects than the impoverished animals.

(2) Evaluate how differentially housed animals are behaviourally and cognitively influenced by the physiological response following psychological stress. Based on their differences in corticosteroid receptor density, the hypothesis was that enriched animals would be more sensitive to stress exposure prior to tests. This would be manifested by increased exploratory behaviour and enhanced learning and memory performance in comparison with the impoverished animals. We also expected to see a stress intensity-dependent effect. Mild stress exposure was expected to enhance memory performance compared to the nonstress and high stress treatments, and this effect would be more pronounced in the enriched animals.

(3) Compare the long-lasting effects of one aversive exposure (shock) between the differentially housed animals. Based on emotional differences in these animals, we

hypothesised that the effects would be more persistent in the impoverished rats.

2. Method

Subjects were 64 male, 3-month-old Sprague–Dawley rats. They were delivered by the commercial breeder Alab, Sollentuna, Sweden. They were maintained on a 12-h on/12-h off lighting schedule (lights on at 0600 h) in a room thermostatically maintained at 22 ± 1 °C. Ad lib food and water was available. After 1-week habituation in standard cages (45 × 30 × 20 cm, four rats per cage), the rats were housed in two differential environmental conditions for 30 days. Thirty-two rats were reared (eight per cage) in large wire mesh cages (100 × 60 × 35 cm) containing a variety of stimuli, i.e., wheels, ladders, tunnels and balls that were daily replaced (enriched environment). The remaining 32 rats were housed singly in individual plexiglas cages (16.5 × 22.5 × 13.5 cm) without any exposure to stimulation (impoverished environment). At the time of behavioural tests, the mean body weight of enriched housed animals were 408 g and impoverished house animals 442 g ($P < .05$). This differential housing effect on body weight is in line with several earlier reports (Renner and Rosenzweig, 1987; Mohammed et al., 1993), and does not appear to be a factor in performance in the water maze. Table 1 presents the schedules of differential housing and behavioural testing.

Table 1
Flowchart illustrating schedule of experimental procedures

Week	Day	Procedure
0		Arrival of animals and habituation week
		Housing in group cages
1–4	1–30	Differential housing (in enriched or impoverished environment)
5–7	31–42	Training and testing period
		Housing in individual cages
	31	60-s free swim trial in Morris water maze (MWM)
	32	Exposure to different treatment conditions (see Table 2 for specification of treatment groups).
	33	Resting
	34	
	35	First open-field test
	36	MWM training (4 trials/day)
	37	
	38	
	39	
	40	Resting
	41	
	42	Retention probe trial in MWM
8–9	42–57	Housing in group cages
9	57	Second open-field test

Arrival of animals was on Week 0. The following week (Weeks 1–4), animals were housed in different environments. The main experimental period was on Weeks 5–7. Retesting in open-field was on Week 9.

Table 2
Description of treatments during the conditioning and reexposing phase

Treatment		Description
CC	Control	No conditioning. Always left undisturbed until testing started.
SC		Shock exposed during the conditioning phase but not reexposed before testing.
EE	Exposed	Only exposed to the apparatus as conditioning and before testing.
SE		Shock exposed during the conditioning phase and reexposed before testing.

When revealing the effect from stress per se, the control group consisted of the CC + SC animals and the expo group consisted of EE + SE animals.

Following the 30-day period of differential housing, all rats were placed in individual plexiglas cages and remained in these cages during the experimental period. However, after the retention probe test in Morris water maze (see below), they were again housed in standard cages and remained there for 15 days before being retested in the open-field. During this period, the same four animals shared cages as they did during the habituation week. Consequently, animals from different environment and treatment groups shared cages during this period (for the different groups tested, see Table 2).

The experiments were conducted between 0800 and 1200 h each day. During the experimental session, all rats were placed in a soundproof room, and they were moved there at least 90 min before any experimental activity started. The differentially housed rats were divided into four treatment groups (eight rats per group). They were as follow: control–control (CC), exposure–exposure (EE), shock–control (SC) and shock–exposure (SE) (see Table 1 for further details). Thus, there were three stress-exposed groups, with the EE being considered as the mild stress group. The exposures were made prior to the first session. The test order protocol was prepared so that animals from each environmental and treatment condition were tested in blocks of eight rats. These blocks were tested in the same order and at approximately the same time each day. The test order for individual animals were randomised in these blocks. Rats, which belonged to the same test block, were well separated from each other. Testing of a block of eight rats in the Morris water maze took approximately 45 min. After training in Morris water maze, all rats were left undisturbed for at least 60 min in the waiting room. This procedure was repeated on 5 consecutive days. No exposure to the stressor occurred before the retention test.

2.1. Stress conditioning

The psychological stress was induced via reexposing the animal to an aversively conditioned environment/box. The

box used for conditioning and reexposure was a passive avoidance learning apparatus. The apparatus consisted of two compartments with a grid floor, which could be electrified separately for each compartment. One compartment ($25 \times 15 \times 19$ cm) was illuminated and comprised of white walls and a plexiglas roof, while the other compartment was nonilluminated and consisted of black walls and a black roof. A guillotine door (6-cm width, 9-cm height) separated the compartments.

Two days before any experimental training or testing started, the rats were first exposed to the apparatus. During the conditioning session, the EE, SE and SC rats were placed in the white compartment with the head pointing away from the guillotine door. During the first 10 s, they were only allowed to explore the illuminated compartment with the door closed. After 10 s, the door was opened and the rats were allowed to enter the dark side. After the rats entered the dark side, the door was closed and the SE and SC groups received a 2-mA footshock for 2 s. After 10 more seconds, the roof of the black side was opened and the rats were gently removed.

Reexposing the SE and the EE animals to the passive avoidance box (white compartment) then served as the stress-inducing factor. On the following days, these animals were reexposed to the apparatus for 90 s and were then returned to the home cage for 15 min before testing started. SC and CC rats were left undisturbed until the test started.

During reexposure, the rats were first placed in the illuminated side. After 10 s, the door was opened and the rats were allowed to explore freely. No shock was delivered. After reexposure, the rats were removed back to the home cage and into the waiting room until the training or testing started.

2.2. Open-field

Motor activity was assessed in automated activity cages, with recording every 5 min for a period of totally 30 min. The apparatus consisted of a plexiglas open-field box ($700 \times 700 \times 450$ mm) equipped with two rows of infrared-sensitive photocells. Interruptions of photocells beams were counted by a microcomputer. Locomotion counts were registered when the low row of photocells was interrupted, while rearing counts were recorded by the higher row of photocells. The numbers of faecal boil were also counted in each arena. On each test, the animals were singly placed in the centre of the activity cages. Four boxes were used simultaneously. Animals from different housing condition and treatments were tested simultaneously and tested in randomised order (randomised in the blocks described above). Between each testing, boxes were cleaned with water followed by 70% ethanol. Twenty-four days after the first open-field test, the animals were given a second open-field test.

2.3. Morris water maze

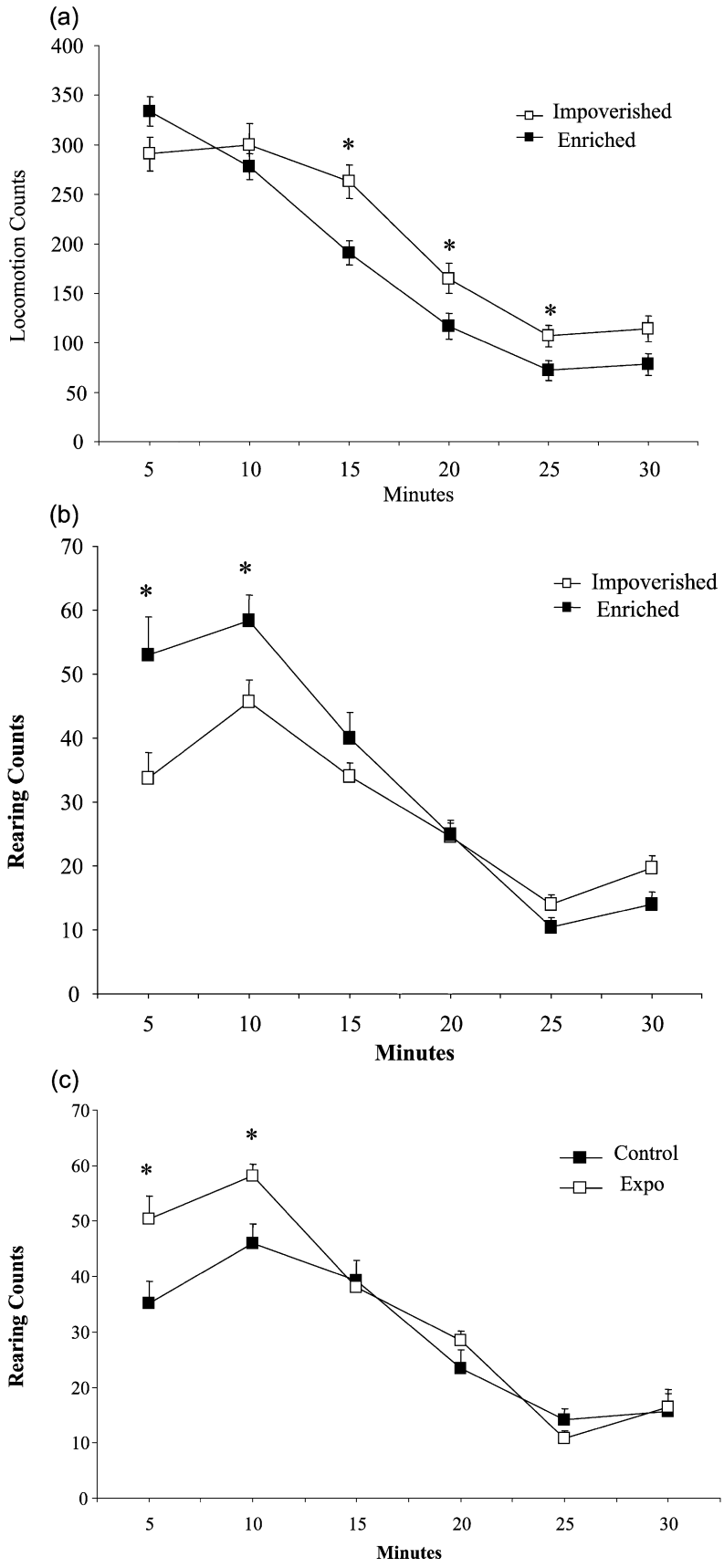
Cognitive function was evaluated in the Morris water maze task (Morris, 1984). This task requires rats to learn the spatial location of a hidden platform in a large circular pool (140-cm diameter) filled with clear water maintained at 21 °C. A transparent platform was placed 2 cm below the surface of the water in a particular position. The rats had the opportunity to use distal cues in order to locate the submerged platform on which it could escape. Latency, distance, speed and swim path travelled to locate the submerged platform were recorded and stored by a PC 386 computer. The decrease in distance swam and latency to find the platform during sequential training sessions was used as measures for spatial learning and memory. The exposure treatments were given prior to the first session of the day.

Before training, the animals were allowed to swim for 1 min. At the beginning of each training session, the animals were placed in the pool facing the wall. Four trials were given each day and four fixed start positions were used. If the rat failed to locate the platform within 60 s, it was placed on the platform and remained there for 30 s until the next trial, i.e., 30-s intertrial interval. The latency value 65 s was given automatically for unsuccessful trials. After the fourth trial, the animal was gently dried with a towel and placed in the home cage back to the waiting room. After 4 training days, the rats were left undisturbed for 2 days. On the third day, they were given one 60-s retention probe test in which the platform was removed from the pool. During retention, the total time each rat swam in the former platform quadrant were recorded, as well as the number of times the animal crossed the former platform position.

2.4. Statistics

Data was analysed by STATISTICA software data program. For determining the effect of differential housings and stress, a multivariate ANOVA for Housing (EC−IC) × Treatment (expo−control) × Repeated Measures (5-min period in open-field, days in Morris water maze) was performed. For determining the effects of different stress intensities, a multivariate ANOVA was performed using Housing × Treatment (CC, EE, SC, SE) × Repeated Measurements, as factors. An identical analysis was performed

Fig. 1. (a) Effect of differential housing on open-field locomotion behaviour. The enriched animals ($n = 32$) showed higher locomotion scores initially and habituated faster than the impoverished animals ($n = 32$). Data presented as mean ± S.E.M. * $P < .05$. (b) Effects of differential housing on open-field rearing behaviour. The enriched animals showed significantly higher rearing scores during the first 10 min of measurement in open-field and habituated faster than the impoverished animals. Data presented as mean ± S.E.M. * $P < .01$. (c) Effects of psychological stress on open-field behaviour. Animals exposed (expo) to a short bout of psychological stress 15 min prior to open-field test showed significantly higher rearing counts during the first 10 min of measurement in the open-field test (expo, $n = 32$; control, $n = 32$). Data presented as mean ± S.E.M. counts. * $P < .05$.


(a)
Locomotion Counts
400
350
300
250
200
150
100
50
0
5
10
15
20
25
30
Minutes
Impoverished
Enriched
*
*
*
(b)
Rearing Counts
70
60
50
40
30
20
10
0
5
10
15
20
25
30
Minutes
Impoverished
Enriched
*
*
(c)
Rearing Counts
70
60
50
40
30
20
10
0
5
10
15
20
25
30
Minutes
Control
Expo
*
*

for determining the effect of shock exposure. The only difference was that shock–control replaced expo–control as treatment factor. When the ANOVA reached statistical significance (which was set at $P<.05$), LSD post-hoc test was used for further analysis. For retention in Morris water maze, data was analysed by two-way ANOVA with Housing × Treatment, as factors.

3. Results

For clarity, the results are presented in the following order (separately for each testing parameter). First, results from enrichment/impoverished comparison and how these animals are affected by stress. Secondly, results on how differences in stress intensities affect these animals. Thirdly, results of shock treatment are presented.

3.1. Open-field

3.1.1. First open-field test

3.1.1.1. Locomotion. There was a significant time effect [$F(5,300)=86.22$, $P<.0001$] indicating habituation to the open-field testing by all groups. There was also a significant Housing × Time interaction effect [$F(5,300)=2.77$, $P<.05$]. Post-hoc test showed that enriched animals had significantly lower locomotion scores during the 15-, 20- and 25-min testing periods (Fig. 1a).

3.1.1.2. Rearing. There was again a significant time effect [$F(5,300)=53.67$, $P<.001$], and a significant Housing × Time interaction effect [$F(5,300)=4.92$, $P<.001$]. Post-hoc analysis showed that enriched animals had significantly higher rearing scores than impoverished animals during the 5- and 10-min testing periods (Fig. 1b). There was also a significant Treatment × Time interaction effect [$F(15,300)=3.73$, $P<.01$]. Post-hoc test showed that the exposed (expo) animals had significantly higher rearing scores during the first 5- and 10-min testing period than the control group (Fig. 1c).

3.1.1.3. Faecal boli. The enriched animals had significantly lower number of faecal boli than the impoverished animals during the open-field test [$F(1,62)=6.07$, $P<.05$] indicating less emotional reactivity to the novel environment.

3.1.2. Second open-field test

3.1.2.1. Locomotion. Reexposing the animals to the open-field revealed a significant main effect of housing [$F(1,60)=4.08$, $P=.05$], and again it was the enriched animals that showed lower locomotor scores than the impoverished animals (Fig. 2). There was also a significant effect of time [$F(5,300)=53.23$, $P<.0001$].

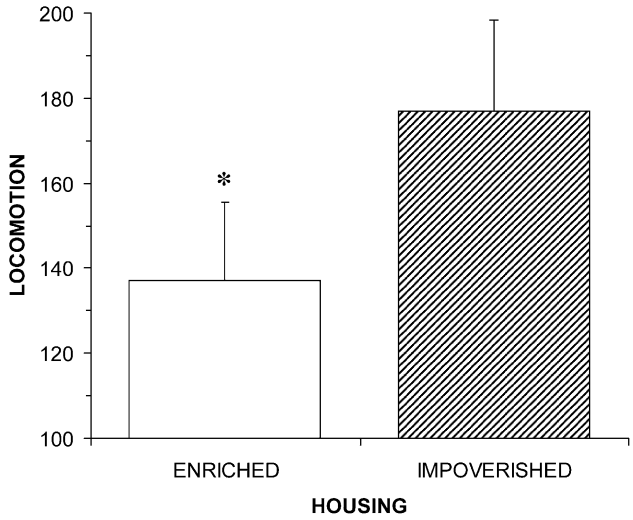


Fig. 2. Effects of differential housing on locomotion behaviour during the second open-field test (22 days after the first open-field test). The enriched animals ($n=32$) showed lower locomotion scores for the total time of measurements (30 min) than the impoverished animals ($n=32$). Thus, the enrichment effects on emotional reactivity are long-lasting and persist even after extensive behavioural tests. Data presented as mean of 5 min ± S.E.M. * $P<.01$.

3.1.2.2. Rearing. The only significant effect that appeared was for time [$F(5,300)=24.21$, $P<.0001$].

3.2. Morris water maze

3.2.1. Training period

3.2.1.1. Latency. There was a significant Housing × Treatment × Day interaction effect [$F(3,180)=4.05$, $P<.01$]. Post-hoc test showed that on Days 1 and 2, the enriched expo group had significantly shorter escape latency than their control group, and both the impoverished groups (Fig. 3). By contrast, on the same days, the impoverished expo group had longer escape latency than their control group. Furthermore, on Day 2, the enriched control group had shorter escape latency than the impoverished expo group (Fig. 3). On Day 4, the enriched expo group had significantly longer escape latency than both the impoverished expo and control groups.

There was a main effect of housing [$F(1,60)=4.07$, $P<.05$] and days [$F(3.180)=120.84$, $P<.0001$], as well as a Housing × Days interaction effect [$F(3,180)=7.39$, $P<.0001$] on escape latency, which was due to the enriched animals having significantly lower escape latencies on Days 1 and 2. There was also a significant Housing × Treatment effect [$F(1,60)=8.39$, $P<.01$], which was due to the impoverished expo animals having longer escape latencies than their control group, and they took longer time than the enriched expo group (Fig. 3).

3.2.1.2. Distance swum. There was also a three way interaction effect of Housing × Treatment × Days [$F(3,180)=$

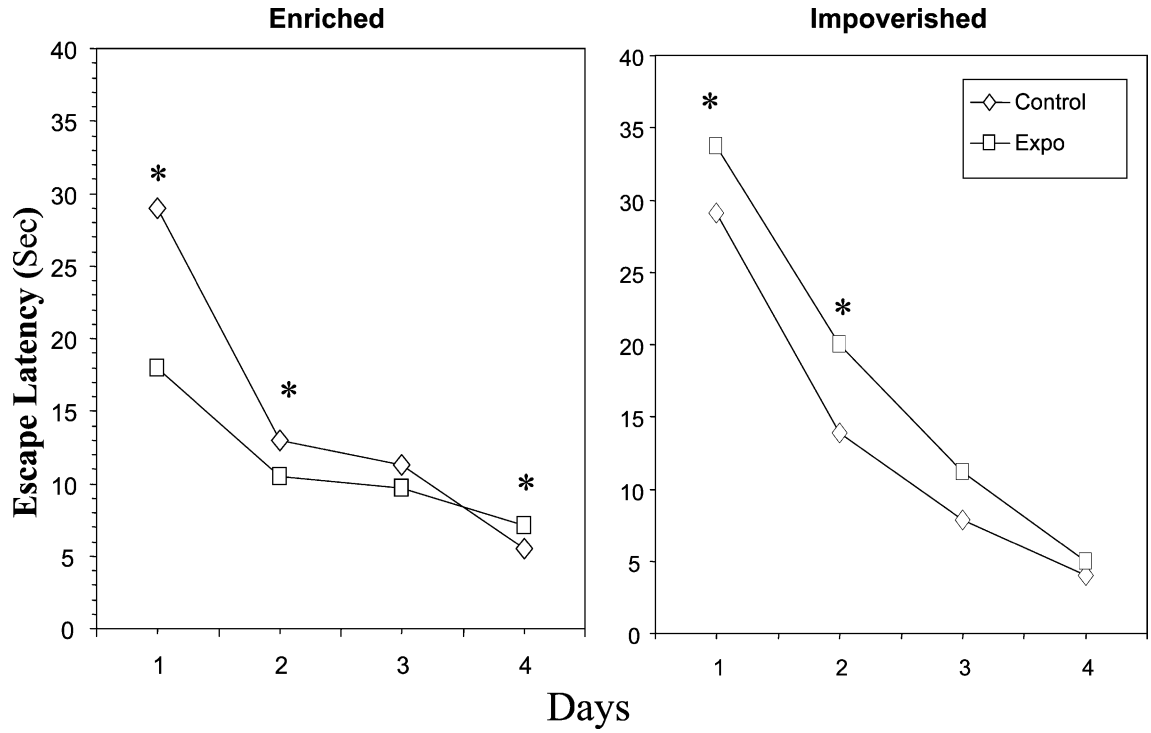


Fig. 3. Spatial learning following stress exposure in differentially housed rats. The enhancing effects of stress expo in enriched animals ($n=16$) and the impairments seen in the impoverished animals from the same treatment ($n=16$) were most pronounced during the first days of testing and then diminished over days. Data are presented as mean escape latency (s). * $P<.01$ (expo compared to control, $n=16$ for each housing condition).

3.20, $P<.05$]. The main effect of housing approached significant level [$F(1,60)=3.43$, $P=.07$]. There was a significant main effect of days [$F(3,180)=155.95$, $P<.0001$] and a significant interaction effect of Housing × Treatment [$F(1,60)=7.32$, $P<.01$], Housing × Days [$F(3,180)=6.41$, $P<.001$], Treatment × Days [$F(3,180)=3.25$, $P<.05$]. Since the results were similar to those described for latency, they are not further presented here. The only difference from the latency data was the Treatment × Day interaction. Post-hoc test of this effect showed that on Day 1 of testing the expo animals swam significantly shorter distance than the control animals.

3.2.1.3. Speed. There was a main effect of housing [$F(1,60)=3.80$, $P<.05$], treatment [$F(3,60)=10.12$, $P<.01$] and day [$F(3,180)=10.69$, $P<.0001$] on speed. The enriched animals swam significantly faster than the impoverished animals, and the expo animals swam slower than the control animals. There was also a Housing × Day interaction effect [$F(3,180)=20.97$, $P<.0001$]. Post-hoc analysis revealed the enriched animals swam significantly slower than the impoverished animals on Day 1 and swam significantly faster on the remaining 3 days. (Fig. 4).

3.2.1.4. Retention test: Morris water maze. There was a significant treatment effect on latency to cross the former platform position [$F(1,60)=5.90$, $P<.05$]. The exposed animals had significantly longer latency to reach the former platform position than the control animals (Fig. 5). There was a significant effect of housing on speed [$F(1,60)=5.92$, $P<.05$] and distance swam to locate the previous platform position [$F(1,60)=5.83$, $P<.05$]. Post-hoc tests showed that the enriched animals swam significantly faster and longer than the impoverished animals.

Thus far, we have described the analysis on enriched/impoverished groups and to what extent psychological stress and the concomitant physiological reactions affect them. As hypothesised, the enriched animals performed better in the behavioural and cognitive tests, and it is obvious that they are differentially affected by stress in comparison to the impoverished animals. The next question was whether this housing-dependent effect of stress could be differentially modulated by variations in stress intensity. To evaluate this, the data was split into the four treatment groups (CC, SC, EE and SE) for each housing condition. The results from this analysis were as follows.

In the open-field test, there was a significant Treatment × Time effect [$F(15,280)=3.96$, $P<.0001$] for locomotion. Post-hoc analysis revealed that SC and SE groups had significantly lower locomotion scores than CC and EE groups during the first 5-min testing periods (Fig. 6a).

There was also a significant Treatment × Time interaction effect [$F(15,280)=4.84$, $P<.0001$] for rearing. Post-hoc test showed that SC groups had significantly lower rearing scores during the first 5-min testing period than all the other groups. During the same time period, the SE group had significantly lower rearing scores than the EE group (Fig. 6b).

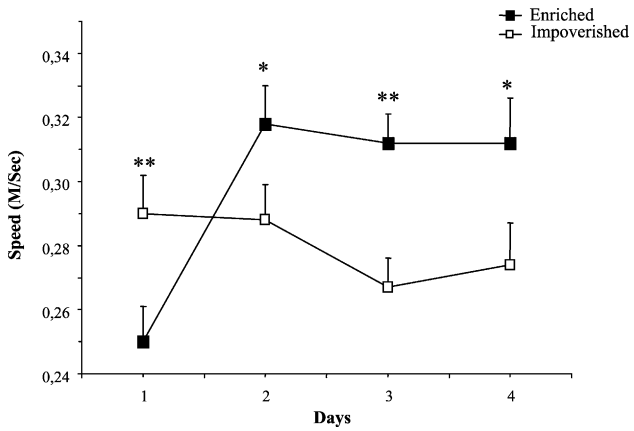


Fig. 4. Effects of differential housing on swim speed. The enriched animals ($n=32$) swam significantly slower than the impoverished animals ($n=32$) on Day 1 of testing, and then they increased their speed and swam significantly faster ($P<.001$) on the remaining days. This difference in swim speed can reflect differences in exploratory behaviour and search strategies between the differentially housed animals (see text for further details). Data are presented as mean speed (m/s) ± S.E.M. * $P<.01$; ** $P<.001$.

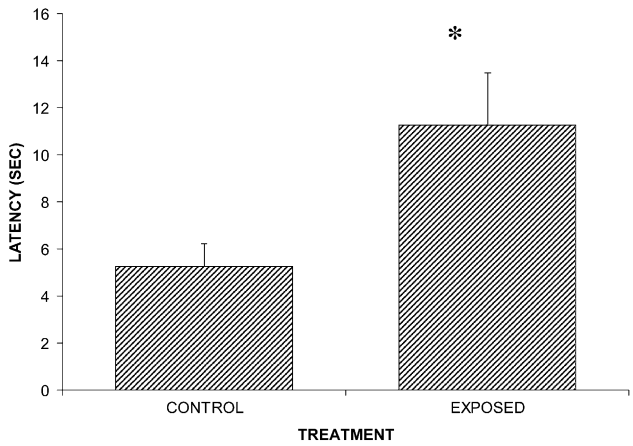


Fig. 5. Effect of stress exposure prior to training in Morris water maze. Stress preexposure impaired long-lasting memory (3 days) in animals independent of housing experience ($P<.05$). Thus, expo animals ($n=32$) took significantly longer time to locate the former platform position compared to control ($n=32$). Data are presented as mean latency (s) ± S.E.M. * $P<.05$.

3.2.1.5. Second open-field test. There was a significant Treatment × Time interaction effect [$F(15,275)=2.55$, $P<.01$] for locomotion. Post-hoc tests revealed that the SE had significantly lower locomotion scores than the other groups during the first 5-min of testing (Fig. 6c).

Similarly, for rearing, there was a significant Treatment × Time interaction effect [$F(15,280)=3.00$, $P<.001$] during reexposure to the open-field test. Post-hoc tests showed that SC and SE groups had significantly lower rearing scores than the CC and EE groups during the first 5-min testing period (Fig. 6d).

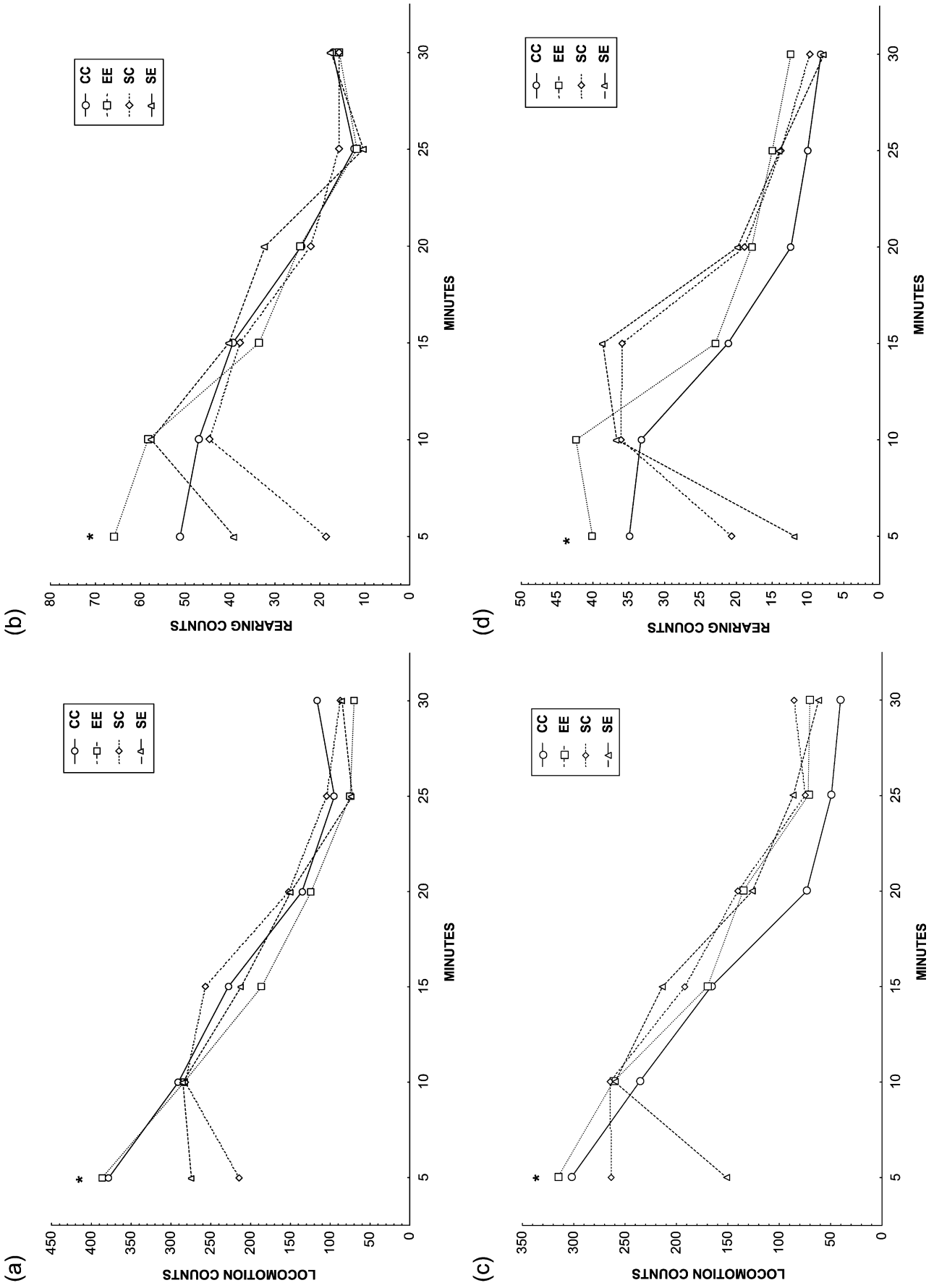
3.2.2. Morris water maze

3.2.2.1. Training period. There was a significant three-way interaction effect of Housing × Treatment × Day [$F(9,168)=2.52$, $P<.01$]. Post-hoc tests showed that, on Day 1, the enriched CC groups had significantly longer escape latency than the enriched EE and SE groups and, on Day 2, they had longer escape latency than the enriched EE and SC groups (Fig. 7). On Day 3, the enriched EE group had significantly shorter escape latency than the enriched SE group. In impoverished animals, none of these performance-enhancing effects of exposure were seen. Rather, their performance appeared to be impaired by this exposure (Fig. 7). Thus, for animals from the impoverished environment, the SE group had significantly longer escape latency on Day 1 than the impoverished SC group and, on Day 2, they had longer latency than the impoverished CC group. On Day 3, the impoverished SE group had significantly longer latencies than all the other impoverished groups (Fig. 7).

There was a significant two-way interaction effect for Housing × Treatment [$F(3,56)=3.40$, $P<.05$] on escape latency to locate the submerged platform. Post-hoc analysis revealed that the enriched CC group had significantly longer escape latency than the enriched EE group. On the other hand, the impoverished CC and SC groups had significantly shorter escape latency than the impoverished SE group.

Further post-hoc comparison revealed differences between the treatment groups from different housing conditions. On Day 1, enriched EE group had significantly shorter escape latency than the impoverished EE group (Fig. 7), and the enriched SE group had significantly shorter

Fig. 6. (a) Effect of differences in stress intensity and of shock on locomotion behaviour in the open-field test. Shock exposed animals (SE $n=16$ + SC $n=16$) showed less locomotor activity during the first 5 min of measurement in the open-field test, in comparison to nonshocked animals (EE $n=16$, CC $n=16$) ($P<.0001$). This behavioural inhibition effect of shock treatment was also confirmed by separate analysis of shock that revealed a main effect of this treatment ($P<.001$) (data not shown). * Indicates time where significant effects appeared, see text for details. For clarity, error bars are not shown. (b) Effect of differences in stress intensities and of shock on rearing behaviour in the open-field test. The SC ($n=16$) animals showed lower rearing scores during the first 5 min period compared to the other groups (SE $n=16$, EE $n=16$, CC $n=16$) ($P<.0001$). This result shows that exposure to a single session of traumatic event can inhibit exploratory behaviour later in a risky situation. * Indicates time where significant effects appeared, see text for details. (c) Effect of stress intensities and of shock on locomotion behaviour during reexposure to the open-field arena. The SE animals ($n=16$) showed less locomotor scores during the first 5 min of measurement, in comparison with the other groups (SC $n=16$, EE $n=16$, CC $n=16$) ($P<.01$). * Indicates time where significant effects appeared, see text for details. (d) Effect of stress intensities and of shock on rearing behaviour during reexposure to the open-field arena. Both the SE ($n=16$) and the SC ($n=16$) groups reared less during the first 5 min of measurement compared to the nonshocked animals (EE $n=16$, CC $n=16$) ($P<.01$), indicating a long-lasting behavioural inhibition effect after shock treatment. * Indicates time where significant effects appeared, see text for details.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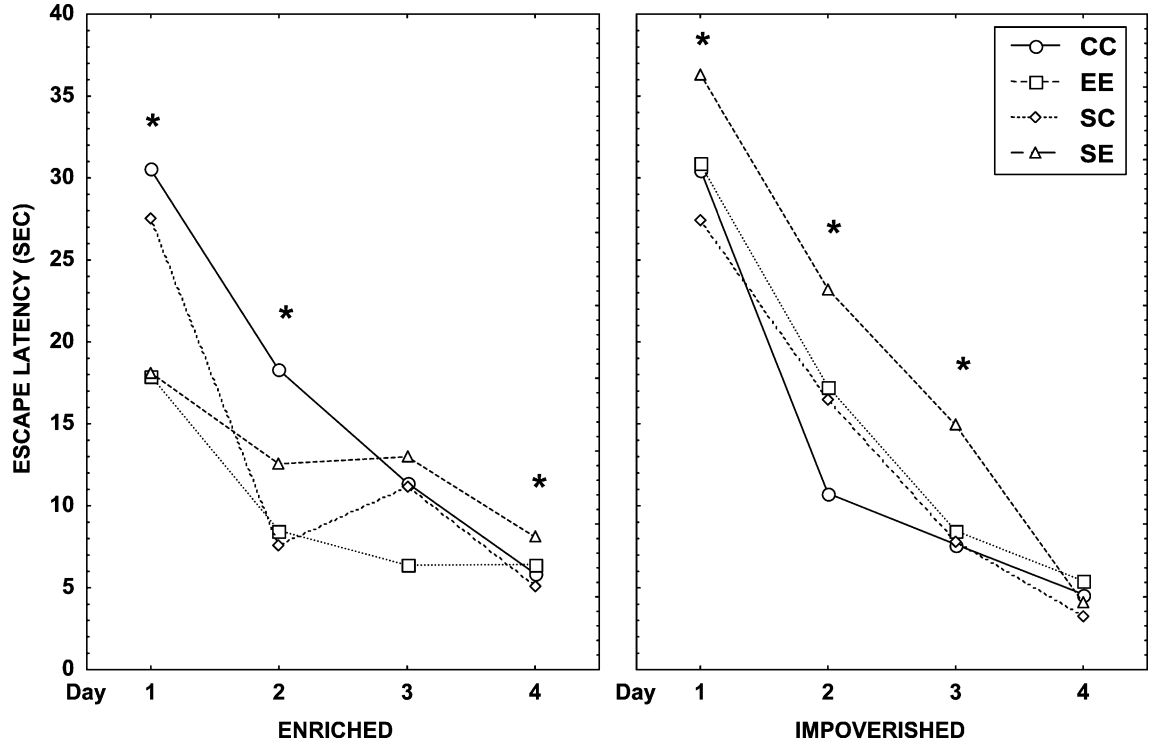

Fig. 7. Effect of preexposure to stress in differentially housed rats on acquisition in Morris water maze. The enriched EE ($n=8$) and SE ($n=8$) groups had shorter escape latency on Days 1 and 2 of training compared to enriched CC ($n=8$) ($P<.05$), indicating that stress exposure prior to training facilitates exploration and acquisition in these animals. The impoverished animals showed quite opposite pattern, whereby the SE animals ($n=8$) were impaired in comparison with all the other impoverished groups (SE $n=8$, EE $n=8$, CC $n=8$) on the first 3 days of testing ($P<.05$). On Days 3 and 4 of training, the SE animals ($n=8$) were impaired in comparison with the enriched EE group on Day 3 ($P<.05$) and in comparison with all the other enriched groups on Day 4 ($P<.05$). Hence, enriched animals are more positively affected by the mild stress in comparison with the impoverished animals. (See text for further details.) Data are presented as mean latency (s). * $P<.05$ (expo vs. control in respective housing conditions).

latency than impoverished SE group. This finding was also evident on Day 2, although for the EE groups the difference just missed the statistical significance ($P=.06$). There was also a trend for enriched SC group to have shorter escape latency than the impoverished SC animals ($P=.06$). On Day 4, the enriched SE group had significantly longer latency than the impoverished SE group.

For distance swum to locate the platform, there was a significant three-way interaction effect of Housing × Treat-Treatment × Day [$F(9,168)=2.55$, $P<.01$]. Post-hoc tests showed that the enriched CC group swam significantly shorter distance on Day 1 than the enriched EE and SE groups, and on Day 2 than the enriched SC group. On Day 3, the enriched EE group swam significantly shorter distance than the enriched SE group, and tended to swim shorter distance than the enriched CC group ($P=.07$). No effects of stress intensity levels were seen in the impoverished animals for different days.

There was a significant Housing × Treatment interaction [$F(3,56)=4.11$, $P<.01$]. Post-hoc tests revealed that the enriched CC group swum significantly longer distance than the enriched EE group. Further post-hoc comparisons between the treatment groups from differentially housed rats revealed similar results as those on latency and are not further presented here.

During the water maze retention test, the results for speed revealed a significant main effect of treatment [$F(3,56)=5,87$, $P<.01$]. Post-hoc test showed that the SE groups swam significantly slower than the CC and SC groups, and

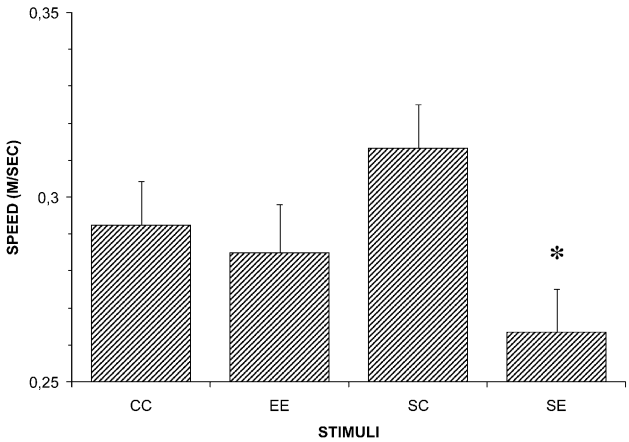

Fig. 8. Effect of stress preexposure on swim speed. The SE-treated animals ($n=16$) swam significantly slower than the SC group ($n=16$) when searching the submerged platform ($P<.05$). Data are presented as mean speed (m/s) ± S.E.M. * $P<.05$ (SC compared to SE).

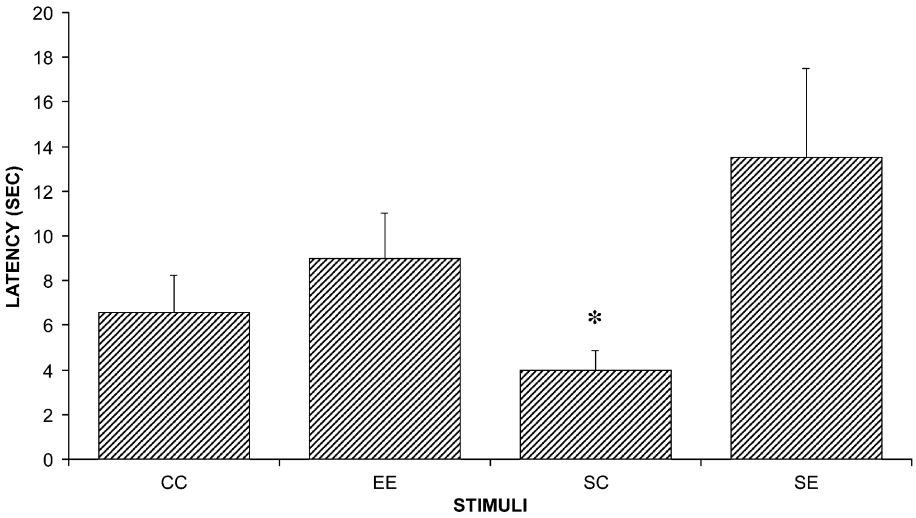

Fig. 9. Effect of stress preexposure on retention in Morris water maze. The SC animals were significantly faster to cross the former platform position compared with the SE animals ($P<.05$). Data are presented as mean latency (s) ± S.E.M. * $P<.05$ (SC compared to SE).

they also tended to swim slower than the EE group ($P=.08$) (Fig. 8).

For latency, there was a significant main effect of treatment [$F(3,56)=2.71$, $P<.05$]. Post-hoc analysis showed that the SE group took longer time to cross the former platform position compared to the SC group (Fig. 9).

In conclusion, this analysis of the effects of different stress intensities showed firstly that stress could influence behaviour and cognitive function in an intensity-dependent manner. Secondly, that this modulatory effect of stress intensity could vary depending on the organisms earlier experiences.

In open-field, the analysis of shock treatment revealed a significant interaction effect for Locomotion × Time [$F(5, 300)=9.53$, $P<.0001$] and Rearing × Time [$F(5,300)=9.50$, $P<.0001$]. Post-hoc analysis showed that these differences were during the first 5 min of measurement, in which the shocked animals had significantly lower locomotion and rearing scores than the controls (see also Fig. 8a,b). No differences of significance were seen in the further analysis of shock effects.

4. Discussion

4.1. Housing conditions

The comparison of animals from the differential housing conditions replicates our earlier findings that in the open-field the enriched animals explore more initially and then habituate faster (Mohammed et al., 1986, 1990; Nilsson et al., 1993; Falkenberg et al., 1992; Torasdotter et al., 1996), and that they perform better in the Morris water maze task in comparison to impoverished animals (Mohammed et al., 1986, 1990; Falkenberg et al., 1992; Pham et al., 1999a,b). In the open-field test, in agreement with the findings of Fernandez-Teruel et al. (1992), there was also less faecal boli deposited by the enriched animals indicating less anxiety-related behaviour (Archer, 1973).

In connection to these behavioural effects, the data on swim speed in Morris water maze revealed an interesting pattern. On Day 1, the enriched animals swam significantly slower than the impoverished animals and then they swam significantly faster on the remaining days (Fig. 4). Based on correlations between swim pattern and learning performance and between swim speed and learning performance (Morris, 1984; Oitzl et al., 1994), we interpret these differences in swim speed to reflect divergences in exploratory behaviour. It has been shown that, when animals learn the Morris water maze task, they shift from a nonspecific exploratory behaviour (low speed) to a more goal directed behaviour (faster speed) (Oitzl et al., 1994). Accordingly, the enriched animals utilise a more exploratory search strategy during the first days of testing, and then they shift to more goal directed behaviour as they learn the task (Oitzl et al., 1994). The initial slower swim speed of enriched animals is indicative of the nonspecific search strategy followed by a shift to more goal directed behaviour as revealed by the increased swim speed. This shift in search strategy was not seen in the impoverished animals, which displayed no changes in swim speed.

The enriched animals also showed less locomotion scores than the impoverished animals during the second test in the open-field given 24 days after the first test (Fig. 2), indicating that the enrichment effects on emotionality and exploratory behaviour is long-lasting and also persists after extensive training and testing. At the outset, this long-lasting effect appears discordant to earlier studies, which have shown that the differences in performance between enriched and impoverished animals diminish with cognitive stimulation. As Rosenzweig and Bennett (1996) pointed out, the impoverished animals catch up to the levels of enriched

animals performance in some cognitive tasks. This discordance can be related to the neuronal systems subserving emotional and cognitive directed behaviours (LeDoux, 1996). The subcortical limbic system subserving emotions seems to be especially susceptible to environmental influence during the neonatal period and then to be more rigid later in life, and with increasing age it appears to be more fixed and resistant to environmental influences than the neocortical systems subserving learning and memory. For instance, the emotional consequences of neonatal manipulation are known to persist throughout the life span (Meaney et al., 1988; Fernández-Teruel et al., 1997), while this effect is not observed if the manipulation is given later in life (Denenberg et al., 1967). However, our results show that differential housing for 30 days influence emotionality also in adult animals and that when established this effect is stable over time and can withstand both extensive stimulation during the experimental phase and social housing for 2 weeks thereafter (Fig. 2). The rigidity of the emotional differences could possibly be explained by the fact that this effect, independent of housing conditions, is based on learning and adaptation. The differences between enriched and impoverished animals are to what condition they have adapted.

4.2. Stress and housing condition

The main effect of stress exposure prior to the open-field test was for rearing, where the expo animals explored more than controls during the initial 10 min of measurement (Fig. 1c). This effect was intensity-dependent with animals that received stress of high intensity (SE) showing more rearing activity relative to the mild stressed animals (EE) (see Fig. 6a,b). Thus, it appears that the physiological concomitant of behavioural stress primes for exploratory behaviour in an intensity-dependent manner, i.e., higher stress intensity primes for more exploration.

An interesting comparison here is the striking resemblance between the rearing data from expo–control animals and those obtained from enriched versus impoverished animals (see Fig. 1b,c). The expo animals displayed a similar behavioural pattern as the enriched animals with higher exploratory behaviour initially followed by a rapid habituation. Hence, it seems possible that the higher exploratory behaviour in expo and enriched animals can be mediated by the same mechanism, i.e., corticosteroid receptor activation. However, in the former case, the receptor activation would be due to more ligands being available in the stressed animals, while in the latter it would be due to more receptors that augment the ligand effects. In view of the aims of this study, this finding is interesting, since one of the hypothesis in the present study was that the behavioural and cognitive differences between enriched and impoverished animals could be explained by their differences in their sensitivity to the physiological reaction following stress. The present findings indicate that different sensitivity to corticosterone could be an important factor for mediating the behavioural differences between enriched and impoverished animals. However, since these results of the stress effect per se were not from normally housed animals, they need to be interpreted with caution.

In the open-field reexposure test, the SC and SE animals again diverged from the CC and EE groups by showing less exploration during the first 5 min of testing (Fig. 6c). Since the shock treated animals had lower locomotor scores also during the first exposure, we relate this result both to a persistent effect of shock treatment (anxiety) and to a learning effect. Conceivably, during the first exposure to the open-field, these animals instinctively applied a coping strategy (less exploration), which turned out to be functional, and consequently they invoked the same strategy during the second exposure. Since these long-lasting effects of shock were not seen in our earlier study in standard housed animals (Larsson et al., submitted for publication), it would appear animals from manipulated environmental conditions are more affected by aversive treatments.

Stress exposure prior to training in Morris water maze revealed distinct learning and memory effects in the differentially housed animals. As depicted in Fig. 3, while exposure to stress prior to test enhanced escape performance in enriched animals, the same treatment impaired performance in impoverished animals. These environmentally induced differences were seen following different stress intensity levels. While mild stress (EE) significantly enhanced performance in the enriched animals, it had no impact on escape latency in the impoverished animals. However, after exposure to high-intensity stress (SE), performance on escape latency were severely impaired in the impoverished animals from the first day of training, while in the enriched animals escape performance were only impaired during the last day of training by the same treatment (Fig. 7). As could be expected, these enhancing/impairing effects of stress were especially evident on Days 1 and 2, and then gradually disappeared on Days 3 and 4, as the treatment effect diminished. Thus, mild stress enhanced only the enriched animals escape performance in Morris water maze, while high-intensity stress impaired performance in both groups, with the impoverished animals more affected than the enriched animals. A plausible scenario is that the enriched EE animals' initial superiority reflects a mild stress-induced more efficient exploratory behaviour (Oitzl and de Kloet, 1992; Oitzl et al., 1997), while the impoverished SE animals' impaired performance reflects learning and memory impairments caused by high-intensity stress and corticosteroids (Sandi and Rose, 1994a,b; McEwen and Sapolsky, 1995; Conrad et al., 1999; Lindau et al., 2000; Park et al., 2001).

This thesis is supported by the findings that the SE animals needed longer time than the other groups to reach the former platform position in the retention test in Morris water maze, while the SC animals were faster than the other groups (Fig. 9). Thus, independent of life history, there was

a negative effect on memory performance after exposure to high-intensity psychological stress.

4.3. Shock treatment

In agreement with earlier findings (Dunn and Berridge, 1990; Heinrichs et al., 1992), exposure to shock had a long-lasting inhibitory effect on exploratory behaviour when the animals were exposed to novel situations. However, since this effect was persistent also during the reexposure test in open-field, it seems that it also extends to familiar arenas, which could still be seen as possibly noxious (Figs. 6c,d and 9a,b). An interesting observation is that this effect of shock on emotional reactive behaviour could be attenuated by prior stress exposure. Thus, the SE group had significantly higher rearing scores during the first 5 min of testing in comparison to the SC group. In addition, this effect of higher rearing counts in the SE animals was also evident during the open-field reexposure test, indicating acute as well as long-lasting anti-anxiolytic effects of the physiological response following psychological stress.

4.4. General discussion

The results from this study confirm our hypothesis that experience from enrichment increases reactive exploratory behaviour in novel situations and that enriched animals respond differently to mild stress than do impoverished animals. Furthermore, as discussed above, the striking resemblance between the enriched and the stress exposed animals behaviour initially in open-field and Morris water maze supports the hypothesis that the enrichment effect in part could be explained by a more efficient action of the corticosteroids in these animals.

These suggestions, although rather tentative, find support both from behavioural and psychopharmacological studies. At the behavioural level, these results could be explained by the fact that housing in environmental enrichment induces emotional stability (Mohammed et al., 1993). During the enrichment period, the animals are repeatedly exposed to novel objects, which they are able to explore freely. This would at least initially be comparable to repeated mild stress exposures. Since none of these exposures ever have any aversive outcomes, the inquisitive rats would be primed for excitement and exploration of unfamiliar environment and objects. The repeated mild stress exposure with resultant reinforcement lead to a more emotionally stable organism (Chorpita and Barlow, 1998). This emotionality thesis is also compatible with Renner and Rosenzweig's (1987) postulate that enriched and impoverished animals differ in their susceptibility to risk-taking behaviour, and in the way they acquire environmental information. Thus, the stimulated animals are less fearful to explore new environments from which they can extract information in a more efficient way; hence, their initial high exploratory behaviour and faster habituation. This thesis is also supported by the findings in this study that repeated stress exposures could attenuate the anxiety-like behavioural effect induced by shock (Fig. 6b).

The environmentally induced alteration in reactive explorative behaviour seems also compatible with psychopharmacological investigations of the corticosteroid effects. It has been shown that selective stimulation of MRs decreases reactive exploratory behaviour to objects in novel environments, while this effect was counteracted by stimulation of GRs (Oitzl and de Kloet, 1992). Further, the suppressive effect of MR stimulation on exploration could be counteracted by a selective MR antagonist (Oitzl and de Kloet, 1992; Oitzl et al., 1994, 1997). From these and electrophysiological studies, it was suggested that the MRs activation would influence selection of behavioural strategies and enhance acquisition of information, thus, facilitating learning and memory. By contrast, the GRs, which counteract the MR effect, were supposed to have less behavioural effects and involved in mediating some aspects of spatial memory (de Kloet et al., 1993). However, more recent studies have revealed a more complicated pattern where it has been shown that the behavioural and cognitive effects of corticosterone are dependent on a complex interaction between the MRs and GRs (Conrad et al., 1997, 1999; de Kloet et al., 1993). Thus, enriched animals, which due to higher GR density would be more affected by circulating corticosteroids, explored more initially in the open-field and Morris water maze. These effects on behaviour in open-field and Morris water maze were mimicked, and were also more pronounced after stress exposure in these animals. By contrast, the impoverished animals explored less initially and their behaviour was not altered in the Morris water maze task. Hence, it seems the impoverished animals respond less to low corticosterone levels and, as a consequence, they also habituate slower in comparison to enriched animals. Since the behavioural differences between enriched and impoverished animals are evident also in the absence of prior stress exposure, it would appear that enriched animals are more tonically influenced by resting levels of corticosteroids.

A plausible scenario behind the enrichment effect on reactive and stress-affected behaviour can be as follows. The increased GR expression in enriched animals could provide a more effective negative feedback on paraventricular nucleus in the hypothalamus, and thus inhibiting further secretion of corticotropin-releasing factor (CRF) (see de Kloet et al., 1998 for review). CRF is secreted during stress and does after several stages of physiological interactions trigger corticosterone secretion from adrenal gland. This enhanced feedback will work both during the diurnal variations of resting corticosterone levels and after stress exposure. Hence, these animals would have a more sensitive regulation of resting levels of corticosterone and they would therefore be even more sensitive to changes in circulating corticosterone levels (de Kloet et al., 1993, 1998). That would make them more affected by resting and mild stress levels of

corticosterone. In addition, CRF secretion per se has a well-known anxiogenic effect, i.e., inhibiting exploratory behaviour (Dunn and Berridge, 1990; Heinrichs et al., 1992; Takahashi, 2001). Some of the behavioural effects of CRF antagonists mimic those seen after mild stress exposure and/or enriched housing. Therefore, it is possible that the negative feedback exerted by GRs on CRF secretion could be responsible for some of the behavioural effects seen after differential housing conditions.

These behavioural effects together with the fact that the differences in escape latency were most pronounced on Days 1 and 2 of training lead to questions of major importance regarding the nature of the housing effects on learning and memory. Is it a real learning (consolidation) effect due to the environmentally induced neuroanatomical and neurophysiological differences between differentially housed animals, or does it rather reflect a secondary effect due to environmentally induced differences in reactive and inquisitive behaviour during the training sessions? While the behaviour of course is an effect of the animals neuronal function, the enriched network per se need not necessarily be the primary reason behind the enhanced learning performance (Devenport et al., 1992). However, the increased GR expression in enriched animals is a possible candidate, which directly could influence learning and memory (Meaney et al., 1988; Mohammed et al., 1993). Via increased GR expression and enhanced feedback, the enriched animals will be more responsive to low levels of corticostrone, and hence they will be more susceptible to cognitive enhancement associated with low levels of corticosterone. Due to their enhanced feedback, they will also be less negatively affected by variation in stress levels (Stein-Behrens and Sapolsky, 1992; McEwen and Sapolsky, 1995; McEwen, 1999).

An interesting point here is that, while the behavioural effect of stress exposure and enriched housing were similar, these animals learning and memory abilities were differentially affected. In agreement with our earlier studies (Larsson et al., submitted for publication), there were no main effects of exposure compared to control. However, both the enriched and the impoverished animals were cognitively affected by this treatment. That indicates that the corticosterone effect on cognition and behaviour could be mediated by two separate routes. Possibly, the learning and memory effects is due to GRs directly via influence on consolidation processes, while the behavioural effect is mediated by the concerted actions of MRs and GRs (Oitzl and de Kloet, 1992; Conrad et al., 1999). This explanation is also compatible with the results showing that the effect of high-intensity stress was most pronounced during the later training sessions and during retention in Morris water maze.

The differential actions of corticosteroids on behaviour and neuronal function is an important factor to consider when investigating the nature of the learning and memory differences between enriched and impoverished animals. More studies, including analysis of different hormones, are required to explore the significance of MR and GR diversity in the differentially housed animals and the implication of reactive behavioural differences, as well as its relationship to cognitive function such as learning and memory.

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