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Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats

Nicole C.A. Schrijver[a], Nina I. Bahr[b], Isabelle C. Weiss[b], Hanno Würbel[a,*]

[a]*Institute of Animal Sciences, Physiology and Animal Husbandry, ETH Zurich, Schorenstrasse 16, Schwerzenbach 8603, Switzerland*
[b]*Behavioural Neurobiology Laboratory, ETH Zurich, Schorenstrasse 16, Schwerzenbach 8603, Switzerland*

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Abstract

Male Lister hooded rats were reared from weaning either singly or in groups of three in either barren or enriched cages ($n = 9$ each) to study effects of isolation rearing and environmental enrichment on open-field activity, object exploration, activity in the Light/Dark box (L/D box), spatial learning and memory in the Morris water maze, and hypothalamic–pituitary–adrenal (HPA) activity in response to restraint stress. Regardless of inanimate background, isolation rearing mainly enhanced activity under several conditions of environmental novelty. By contrast, environmental enrichment, regardless of social background, primarily accelerated habituation to novelty and improved spatial learning and memory. None of the treatments significantly altered basal and response levels of plasma ACTH and corticosterone. Furthermore, rats reared singly in barren cages showed persistent activity in the L/D box, indicating an interaction between isolation-induced hyperactivity and reduced habituation due to barren caging. These results show that isolation rearing and environmental enrichment affect behaviour selectively, while at the same time revealing biologically relevant interactions between social and inanimate stimulation. It is concluded that systematic variation of social and inanimate stimulation can help distinguish between effects that generalise across variation in environmental background and effects that are idiosyncratic to a specific environmental background.

Keywords: Rats; Environmental enrichment; Social isolation; Standardization; Behavior; Exploration; Novelty; Learning; Memory; Anxiety; Stress; ACTH; Corticosterone

1. Introduction

Environmental manipulations in rodents have a long tradition in behavioural and brain research as a tool to study biological mechanisms underlying behaviour and to model symptoms of human psychiatric disorders. In particular, Hebb's (1949) concept of experience-dependent plasticity of the central nervous system inspired scientists to manipulate particular aspects of the animals' experience and to search for structural and biochemical changes in the brain that might explain the behavioural changes induced by these manipulations.

Rosenzweig et al. (1961, 1963) were the first to demonstrate that both formal training on complex spatial tasks and living in spatially complex environments (environmental enrichment) altered neurochemistry and brain weight in rats (reviewed by Rosenzweig and Bennett, 1996). Environmental enrichment has remained a relevant manipulation since then, and only recently, there was a resurgence of interest when several studies found evidence for neurogenesis in response to environmental enrichment in a variety of species (reviewed by Van Praag et al., 2000), which might explain its beneficial effects on the course of neurodegenerative diseases (e.g., Huntington's; Van Dellen et al., 2000), ageing (e.g., Kempermann et al., 1998) and recovery from brain damage (Horner and Gage, 2000). Others were more inspired by the classical work on separation by Harlows et al. (1971) to study the effects of early social, rather than inanimate, stimulation on behaviour and brain function. In rats, social isolation from the age of weaning (±21 days) was found to induce a syndrome involving a variety of behavioural and neurochemical changes compared to group-reared controls (Robbins et al., 1996). Several behavioural impairments (e.g., sensory gating, Geyer et al., 1993; attentional selection, Schrijver and Würbel, 2001) closely mimic key behavioural symptoms of schizophrenic patients (Gray

* Corresponding author. Tel.: +41-1-655-74-79; fax: +41-1-655-72-01.
E-mail address: hanno.wuerbel@inw.agrl.ethz.ch (H. Würbel).

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et al., 1991; Braff and Geyer, 1990; Elliott et al., 1998; Pantelis et al., 1999). Therefore, isolation rearing in rats is extensively used as an animal model of schizophrenia (Weiss, 2001).

Our own work on the effects of environmental enrichment and isolation rearing in rats is strongly inspired by these studies, but has a rather different application. In addition to a general interest in environment-dependent plasticity of brain and behaviour, we are mainly concerned with the implications of rodent housing conditions for animal welfare and for the scientific validity of animal experiments (Würbel, 2001). Rodents are increasingly used to study complex brain functions such as learning, memory, attention or anxiety. The high degree of sophistication of this work in terms of experimental manipulations and behavioural endpoints, however, sharply contrasts with the ways in which the animals' environmental background is taken into account.

First, mice and rats are typically kept singly or in small groups in barren laboratory cages that lack many key features of their natural habitats. These conditions impose constraints on behaviour and brain development, resulting in behavioural abnormalities and aberrant brain functions (Benefiel and Greenough, 1998; Würbel, 2001). This raises the possibility that research based on standard housed rodents might sometimes yield pathologic artefacts. Secondly, although behaviour and brain development strongly depend on environmental background, variation of environmental factors rarely forms an integral part of animal experiments. On the contrary, environmental standardisation both within and between laboratories serves to maximise test sensitivity and reproducibility of results across replicate studies (Beynen et al., 2001). Ironically, however, standardisation entails the risk of obtaining results that are idiosyncratic to the particular study design, and systematic variation of environmental background might be needed to distinguish between idiosyncratic artefacts and informative effects (Würbel, 2000, 2002).

The present study aimed to address the latter issue by investigating the effects of variation in environmental background on the outcome of a variety of standard behavioural tests in rats. Thus, environmental background was systematically varied along two dimensions of environmental stimulation, namely social (isolation rearing versus group rearing) and inanimate stimulation (barren cages versus enriched cages). Social and inanimate stimulations were chosen for three reasons. First, both social and inanimate stimulations play an important role during early ontogeny in behavioural and brain development (Greenough, 1975; Hall, 1998; Renner and Rosenzweig, 1987; Robbins et al., 1996; Van Praag et al., 2000). Secondly, both isolation rearing and environmental enrichment have been extensively used as environmental manipulations in many different areas of research (see recent reviews by Hall, 1998; Van Praag et al., 2000), and a large body of literature has accumulated to which the findings can be related. Thirdly, variation in social and inanimate stimulations is most suitable to cover the range of housing conditions used in rodent experiments.

Unfortunately, the term 'environmental enrichment' is only loosely defined, and in much of the relevant literature refers to 'a combination of complex inanimate and social stimulation' (Rosenzweig et al., 1978). Therefore, effects of social and inanimate stimulation are often confounded (Hall, 1998). It has been suggested that it is the interaction of factors, rather than any single element, that explains the effects of environmental enrichment (Van Praag et al., 2000). However, increasing evidence indicates that social and inanimate stimulation can have dissociable effects on selective parts of the brain and selective brain functions, resulting in dissociable behavioural profiles (Würbel, 2001; Schrijver et al., 2001; Zimmermann et al., 2001). Thus, whereas environmental enrichment induces structural and biochemical alterations mainly in the cortex and hippocampal formation, resulting in enhanced learning and memory especially in hippocampus-dependent tasks (Van Praag et al., 2000; Würbel, 2001), isolation rearing has been mainly associated with changes in prefrontal cortico-striatal monoamine pathways, which, in addition to the specific impairments in the inhibitory control of behaviour, has been reported to induce enhanced responses to novelty and an anxiogenic profile (Robbins et al., 1996; Hall, 1998; Weiss, 2001; Würbel, 2001). Consequently, when both social and inanimate backgrounds were varied independently, nonadditive effects were usually detected (e.g., Varty et al., 2000; Zimmerman et al., 2001; Schrijver et al., 2001).

However, there are also many conflicting findings for almost every aspect of behaviour. With respect to isolation rearing, this has been extensively reviewed by Hall (1998). Isolation rearing most consistently enhances locomotor activity in a novel open field, though in some studies, rats have also been found to be less active in an open field (e.g., Archer, 1969; Gardner et al., 1975; Gentsch et al., 1981; Holson, 1986). In response to novel objects in an otherwise familiar environment, some studies (e.g., Sahakian et al., 1977; Einon and Morgan, 1976) found that isolation-reared rats were more active and displayed retarded habituation in exploration compared to group-reared rats, whereas others (e.g., File, 1978; Zimmermann et al., 2001) found no difference. More consistently, isolation rearing has been associated with an anxiogenic profile across a variety of tasks, resulting in e.g., a longer latency to enter a novel environment (Einon and Tye, 1975; Zimmermann et al., 2001) and less activity on the open arms of an elevated plus maze (Parker and Morinan, 1986; Wright et al., 1991) or in the centre of a brightly lit open field (Gamallo et al., 1986). Again, however, inconsistent findings have also been reported (Hall, 1998). Moreover, as increased anxiety or fearfulness is often associated with enhanced responses to stressors (e.g., Liu et al., 1997; Caldji et al., 1998), it is noteworthy that no consistent picture has emerged from studies on hypothala-

mic–pituitary–adrenal (HPA) axis functioning. Thus, isolation rearing has been found to increase (e.g., Gamallo et al., 1986), decrease (e.g., Sanchez et al., 1995) or have no effect on basal plasma corticosterone levels (e.g., Holson et al., 1991), and similar inconsistencies were found regarding acute responses to, and recovery from, a variety of stressors (e.g., Gentsch et al., 1981; Hall et al., 2001; Weiss, 2001). Finally, although deficits of isolation-reared rats in spatial learning tasks could often be attributed to noncognitive factors (Hall, 1998), Wade and Maier (1986) found that they were also impaired in spatial acquisition learning in the Morris water maze.

With respect to environmental enrichment, the picture appears more consistent, and the improvements in spatial learning and memory are generally robust (reviewed by Van Praag et al., 2000). Unfortunately, enriched reared rats are rarely tested on tasks other than learning tasks. Thus, there are much fewer data available of enriched reared rats on responses to novelty, anxiety and HPA responses compared to isolation-reared rats. Nonetheless, inconsistent findings have been reported on all of these traits. For example, enriched reared rats were more active in an open field in some studies (e.g., Huck and Price, 1975), but not in others (e.g., Van Waas and Soffié, 1996; Pham et al., 1999), and environmental enrichment was found to have inconsistent effects on several measures of emotionality in the Roman rat lines (Fernández-Teruel et al., 1997). Furthermore, although environmental enrichment, like neonatal handling, has been associated with reduced glucocorticoid receptor expression in the hippocampus (Mohammed et al., 1993), there is no consistent evidence for attenuated stress responses in enriched reared rats (Van Praag et al., 2000).

Clearly, there are many aspects of experimental design that might account for conflicting results of seemingly similar studies. Besides differences in the genetic background of the animals, factors related to the test environment (including the experimenter) and procedural details of the tests are known to affect the outcome of standard behavioural tests (Claassen, 1994; Wahlsten, 2001). However, given the important role played by environmental background and, in particular, by early social and inanimate stimulation on behavioural and brain development, variation of these two aspects of environmental background might be particularly suitable for determining the external validity (or robustness) of behavioural phenotypes, while at the same time revealing biologically relevant interactions between treatments and environmental background (Würbel, 2002). In the present study, social (isolation rearing versus group rearing) and inanimate stimulations (barren cages versus enriched cages) from the age of weaning (±21 days) were varied independently using a 2×2 factorial design, to examine the effects of isolation rearing and environmental enrichment on open-field activity, novel object exploration, short-term recognition memory for spatial and object change, activity in the Light/Dark box (L/D box), spatial learning and memory in the Morris water maze, and basal and response measures of HPA activity in response to restraint stress. This was done with the aim to distinguish between effects of either manipulation that generalise across the two variants of the other treatment factor (inanimate or social stimulation, respectively) and effects that depend on an interaction of the two treatment factors. Whereas the former reflects more robust effects of either manipulation that are likely to generalise across a range of environmental conditions, the latter is indicative of effects that are likely to depend on a more specific combination of environmental factors.

2. Method

2.1. Animals and housing conditions

Subjects were 36 males derived from Lister hooded rats obtained from Harlan (Horst, the Netherlands), which were bred and raised at the ETH Research Unit (Schwerzenbach, Switzerland). At weaning (21 days of age), from each of nine litters, four males were assigned to the four housing treatments—‘‘isolate in barren cage’’ (IB), ‘‘isolate in enriched cage’’ (IE), ‘‘group in barren cage’’ (SB), ‘‘group in enriched cage’’ (SE)—according to a 2×2 factorial design with inanimate (barren versus enriched cage) and social background (isolation versus group rearing) as factors, so that each treatment group was counterbalanced for preweaning background.

IB rats were housed singly in Makrolon Type III cages ($27 \times 48 \times 20$ cm) and SB rats in randomly composed groups of three in Makrolon Type IV cages ($38.5 \times 59 \times 20$ cm) containing sawdust as bedding. IE and SE rats were housed either singly or in groups of three in former rabbit cages ($62 \times 70 \times 75$ cm) made of stainless steel with a grid front door carrying the feeder and water bottle. Enriched cages were furnished with a thick layer of bedding material (wood chips and sawdust), offering the rats the opportunity to dig burrows. They contained shelves at different heights connected by wooden branches, hay, a rope, plastic tunnels and a hut made of an opaque Makrolon Type II cage. Barren cages were replaced weekly by new cages, whereby a handful of the old sawdust was added to the fresh sawdust to reduce novelty. In the enriched cages, dirty bedding material and hay were removed weekly and replaced by fresh material, the shelves were cleaned from faeces, objects were partly rearranged and one item (e.g., a toy) was replaced by a new one.

All animals were maintained on a 12-h light/dark cycle (lights on at 0700 h) and had free access to food (Nafag, 9431; Nafag Ecossan, Gossau, Switzerland) and water throughout the rearing period. Breeding, care and all experimental manipulations were conducted in accordance with the Swiss Federal Regulations for animal experimentation and were formally approved by the Swiss Federal Veterinary Office.

2.2. Experimental design

Testing started at 12 weeks of age. Immediately prior to testing, all rats were habituated to an individual transport cage with a sawdust bedding. Subjects were first tested on a series of six consecutive tasks of open field and object exploration, followed by a single session in a L/D box and, finally, 7 days of training and testing of spatial and cue navigation in a Morris water maze. After behavioural testing was completed, all rats were exposed to a standard stressor (20-min restraint) with small blood samples being taken before and at several time points after the onset of restraint to determine basal and response levels of ACTH and corticosterone.

2.3. Open field and object exploration

2.3.1. Apparatus

Exploratory behaviour was assessed in four adjacent square arenas ($76.5 \times 76.5 \times 49$ cm each) made of dark grey plastic, which were located in an experimental room indirectly illuminated by low light (20 lx). Behaviour was recorded by a camera mounted on the ceiling and connected to a video recorder and a video tracking motion analysis system (Ethovision; Noldus Information Technology, Wageningen, the Netherlands). Two different types of objects were used for object exploration: ceramic plant pots (11 cm in diameter, 10 cm high) and disposable plastic tubes (50 ml, 12 cm high) filled with sand (to make them heavier). On each trial, new exemplars were used to avoid object recognition by odour trails left on the objects in previous trials.

2.3.2. Test procedure

Subjects were tested in squads of four littermates from the four different housing treatments. Each of them was placed in one of the four arenas. On each of three consecutive days, three squads of four littermates were tested. The test procedure consisted of six trials of 10 min each with an intertrial interval of 10 min during which the subjects were returned to their individual transport cage.

In the first trial (T_1), subjects were exposed to the empty arena to assess locomotor activity in a novel open field. In Trial 2 (T_2), two objects of the same type (pots or tubes) were placed halfway between the ends of two adjacent sides at 15 cm distance from the arena walls (see Fig. 1) to assess novel object exploration in a familiar open field. In Trial 3 (T_3), two novel exemplars of the same object type were placed in the same positions as in T_2 to assess habituation to the now-familiar objects in a familiar open field. In Trial 4 (T_4), two novel exemplars of the same object type were placed in the arena, with one being displaced to the opposite wall compared to the previous trials (T_2 and T_3), to assess spatial novelty recognition (Fig. 1). In Trial 5 (T_5), two novel exemplars of the same object type were placed in the same positions as in T_4 to assess habituation to the now-familiar spatial novelty. In Trial 6 (T_6), one novel exemplar of the same object type and one exemplar of the other object type were placed in the same positions as in T_5 to assess object novelty recognition (Fig. 1). Half of the subjects of each treatment group ($n = 5$) started with pots; the other half ($n = 4$) with tubes as novel objects.

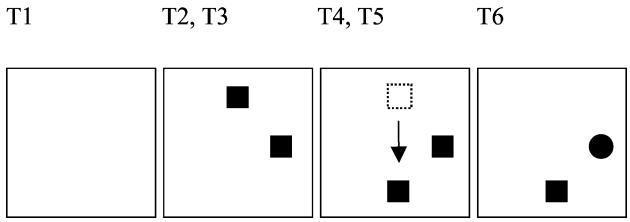

Fig. 1. Schematic representation of the series of six consecutive exploratory trials in the open-field arena (T_1–T_6). In T_1, rats were exposed to an empty novel open field. In T_2, two novel objects of the same kind were introduced. T_3 was a replication of T_2. In T_4, one of the two objects was displaced to a new position. T_5 was a replication of T_4. In T_6, one of the two objects was replaced by a new object of a different kind. Each trial lasted for 10 min, with an intertrial interval of 10 min.

2.3.3. Data recording

All behavioural data were recorded by indirect observation from video recordings using the Observer software (Noldus Information Technology). This included time spent 'moving' (locomotor activity), 'rearing' (standing on hind legs), 'not moving' (sitting or grooming) and 'object exploration' (head and/or forelimbs in direct contact with object or head within 2 cm of object), with all of these behaviours being mutually exclusive. 'Total activity' was calculated by subtracting time 'not moving' from total time.

2.4. L/D box

2.4.1. Apparatus

The L/D box consisted of two equally sized chambers ($29 \times 29 \times 29$ cm each) separated by a wall with a small hole (7 cm in diameter, 7 cm above floor). It was made of wood with a Plexiglas top, with the light chamber painted white with a clear, transparent top and the dark chamber painted black with a black, opaque top. Four adjacent L/D boxes were used. The light chamber was brightly illuminated (120 lx in the centre of each light chamber). Behaviour in the light chamber was recorded by a camera mounted on the ceiling and connected to a video recorder.

2.4.2. Behavioural procedure

Subjects were tested in squads of four littermates from the four different housing treatments. Each of them was placed in the light chamber of one of the four L/D boxes, facing the entrance to the dark chamber. The test lasted for 10 min.

2.4.3. Data recording

Behavioural data were recorded by indirect observation from video recordings using the Observer software (Noldus Information Technology). This included latency to enter the

dark chamber, latency to reenter the light chamber, frequency of crossings between the two chambers and time spent in each chamber. In addition, in the light chamber, times spent 'moving', 'rearing' (standing on hind legs) and 'not moving' (sitting or grooming) were recorded (the black, opaque top prevented insight into the dark chamber).

2.5. Morris water maze

2.5.1. Apparatus

The water maze consisted of a circular tank (2 m in diameter, 60 cm high, bottom 45 cm above floor level) made of black Fiberglas, placed in an experimental room ($3.4 \times 4.9 \times 2.9$ m) containing a variety of distinct extra-maze visual cues. The tank was filled with tap water to a level 30 cm below the rim, which was changed daily and maintained at 21 ± 1 °C. A black circular platform (11 cm in diameter) with a rough surface to facilitate climbing out of the water was placed in either of four virtual quadrants (NE, SE, SW, NW) at 30 cm from the wall. The platform was submerged 2 cm below water level during spatial navigation and marked by a stick (20 cm) mounted on the platform during cue navigation. For probe trials, the platform was removed. Eight equally spaced points at the wall of the tank were designated as N, NE, E, SE, S, SW, W, NW and were used as release points. Swim path was recorded by a camera mounted above the centre of the pool and connected to a video recorder and a video tracking motion analysis system (HVS Image, England, UK).

2.5.2. Behavioural procedure

Subjects were first trained on six consecutive days with four daily trials to locate the hidden platform in a spatially fixed position in order to assess spatial navigation learning. For half of the rats of each treatment group ($n = 5$), the platform was placed in position SE; for the other half ($n = 4$) in position NW. On Day 7, rats were trained for four trials with the platform visually cued and randomly placed in one of the four quadrants to control for any visual impairment. On Days 2, 4 and 6, a probe trial with the platform removed preceded the normal training trials to assess memory formation for the trained platform position. Training trials lasted for 90 s and started with the rat facing the wall of the tank, whereby release point was varied pseudo-randomly. Rats that did not find the platform within 90 s were guided to it by the experimenter. After 30 s on the platform, rats were placed in a warm transparent plastic bucket for an intertrial interval of 30 s. Probe trials lasted for 60 s, whereby rats were released opposite the trained platform position. Following the last trial of a daily session, rats were gently rubbed dry with a towel and returned to their individual transport cages.

2.5.3. Data recording

Swim path of normal training trials was analysed for path length (cm), swim speed (cm/s) and escape latency (s). Probe trials were analysed for time spent in each quadrant (s).

2.6. Restraint stress

2.6.1. Apparatus and procedure

Rats were transported one by one by a familiar experimenter from the colony room to the adjacent experimental room, and bled (time $t_0 = 0$ min) from the tail [0.1–0.2 ml collected into prechilled ethylenediamine tetraacetic acid (EDTA)-coated tubes] (Microcuvette; Sarstedt, Sevelen, Switzerland) by tail incision (Fluttert et al., 2000) within 2 min from entering the colony room to obtain a basal blood sample for analysis of plasma ACTH and corticosterone. Subsequently, the rats were placed in a transparent Plexiglas restraint tube (5 cm in diameter) of adjustable length for 20 min to induce an intense and persistent stress response. After 20 min, they were bled from the same tail incision for a second time (t_{20}) to obtain blood samples for analysis of the acute stress response before release from restraint and transport back to the colony room. At times t_{80} and t_{140}, rats were again transported to the experimental room, bled from the same tail incision for a third and fourth time to obtain blood samples for analysis of recovery from the stressor, and returned to the colony room. On each of three consecutive days, three subjects of each treatment group were sampled in such a way that rats housed together in one cage were sampled on different days.

2.6.2. Plasma ACTH radioimmunoassay

Plasma immunoreactive ACTH titres were quantified using an ACTH ^{125}I radioimmunoassay kit for the determination of human ACTH in EDTA plasma (DiaSorin, Stillwater, MN). To increase assay sensitivity and reduce the volume of plasma needed per measurement, the supplied assay protocol was slightly modified. ^{125}I tracer (50 μl) and 50 μl of antiserum were added to 150-μl aliquots of the five standards (diluted 1:6 in distilled water to give concentrations of 4–120 pg/ml), to 150-μl aliquots of two controls (diluted 1:6 in distilled water) and to 150 μl of sample (diluted 1:10 in distilled water). Standards and controls were measured in triplicate; samples in duplicate in borosilicate glass tubes. Tubes were vortexed and incubated at 4 °C for 20 h. Separation was achieved by adding precipitating complex (250 μl), diluted 1:2 in distilled water, to all tubes except total count. Following brief vortexation and 20-min incubation at 20–25 °C, tubes were centrifuged at $1500 \times g$ for 20 min. The supernatant was aspirated and samples measured in a gamma scintillation counter (Minaxi γ; Packard, Downers Grove, IL), 3-min count per tube. The ^{125}I radioimmunoassay was validated for ACTH in rat EDTA plasma. Interassay precision was 7.6% at 72–85% binding ($n = 6$) and 12.2% at 44–60% binding ($n = 6$), and intraassay precision was 9.3% at 33–42% binding ($n = 6$). Assay sensitivity was 0.2 pg/tube at 95% binding.

2.6.3. Plasma corticosterone radioimmunoassay

Plasma immunoreactive corticosterone titres were determined using an in-house ^{3}H radioimmunoassay validated for rat EDTA plasma and previously described by Pryce et al. (2001). Interassay precision was 10.6% at 40–51% binding ($n=10$) and 6.4% at 18–23% binding ($n=10$), and intra-assay precision was 1.4% at 26–27% binding ($n=10$). Assay sensitivity was 2.5 pg/tube/250 μl at 95% binding.

2.7. Statistical analysis

All analyses are based on a General Linear Model (GLM; SPSS, Chicago, Illinois) using social background (SOC: isolation rearing and group rearing) and inanimate background (ENV: enriched cages and barren cages) as between-subjects factors.

Analysis of locomotor activity and object exploration in the open field further included trial (TRIAL: T_1–T_6) as a within-subjects factor, and changes in behaviour within each trial were analysed using INTERVAL (four timebins of 150 s) as a within-subjects factor. All statistical analyses of the exploratory tasks are based on $n=35$ subjects, i.e., $n=9$ subjects for treatment groups IE, IB and SB, and $n=8$ subjects for treatment group SE because one rat escaped from the open-field arena on several trials. All other analyses are based on the complete set of $n=36$ rats. Analysis of the data obtained in the L/D box also included INTERVAL as a within-subjects factor. Data (escape latency, path length to platform) obtained in the Morris water maze were first ln-transformed to meet the criteria for using a GLM. Day of testing (DAY: d_1–d_6) was used as a within-subjects factor for the training data, whereas day of probe test (PROBE: d_2, d_4, d_6) was used as a within-subjects factor for the analysis of probe test data. From the endocrinological samples, ACTH, but not corticosterone, data were ln-transformed and time of blood sampling (SAMPLE: t_0, t_{20}, t_{80}, t_{140}) was used as a within-subjects factor. In all cases, significant interactions between main factors were further analysed using appropriate post hoc tests.

3. Results

3.1. Open field and object exploration

3.1.1. Total versus locomotor activity

When rats were exposed to a novel open field (T_1), a significant effect of social background on total activity was

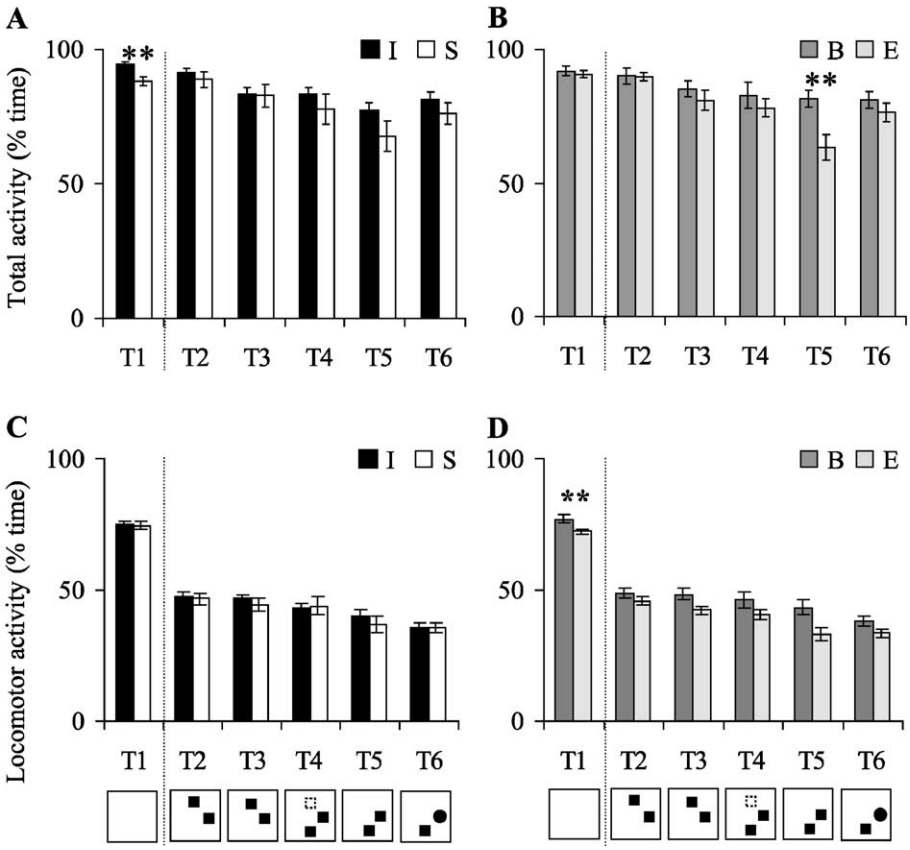

Fig. 2. Activity in each of six consecutive exploratory trials in the open-field arena (T_1–T_6). Upper panels: Total activity (see Method) in percent of time (mean ± S.E.M.) for each trial for isolation- (I = IB and IE pooled) versus group- (S = SB and SE pooled) reared rats (A) and for rats reared in barren (B = IB and SB pooled) versus enriched (E = IE and SE pooled) cages (B). Lower panels: Locomotor activity in percent of time (mean ± S.E.M.) for each trial for isolation- versus group-reared rats (C) and for rats reared in barren versus enriched cages (D). * $P<.05$, ** $P<.01$.

detected [SOC: $F(1,31)=10.71$, $P<.01$], whereas inanimate background had no effect [ENV: $F(1,31)=0.51$, $P>.05$]. Thus, isolation-reared rats (IE, IB) were more active in the novel open field compared to group reared rats (SE, SB), regardless of whether they had been reared in barren or enriched cages (Fig. 2). However, when locomotor activity was analysed on its own, there was a significant effect of inanimate background (ENV: $F(1,31)=7.81$, $P<.01$), whereas social background had no effect (SOC: $F(1,31)=0.15$, $P>.05$). Thus, enriched reared rats (IE, SE) showed less locomotor activity compared to rats reared in barren cages (IB, SB), regardless of whether they had been reared singly or in groups (Fig. 2).

When rats were familiar with the open field and under different conditions of object novelty (T_2-T_6), the effect of social background on total activity had disappeared [SOC: $F(1,31)=1.64$, $P>.05$]. By contrast, there was a strong effect of inanimate background on locomotor activity across T_2 to T_6 [ENV: $F(1,31)=6.15$, $P<.05$]. Thus, reduced locomotor activity in enriched reared rats (IE, SE) compared to rats reared in barren cages was consistent across trials (Fig. 2).

Due to object exploration, locomotor activity was reduced by about 50% on average in trials T_2-T_6 (Fig. 2). Both total activity [TRIAL: $F(4,124)=13.17$, $P<.001$] and locomotor activity [TRIAL: $F(4,124)=16.81$, $P<.001$] decreased gradually across trials. Whereas social background had no effect on the rate of between-trial habituation of total activity [TRIAL × SOC: $F(4,124)=1.06$, $P>.05$], total activity decreased faster across trials in enriched reared rats compared to rats reared in barren cages [TRIAL × ENV: $F(4,124)=3.83$, $P<.05$], resulting in a significant difference in T_5 [$F(1,31)=12.44$, $P<.01$] (Fig. 2).

3.1.2. Object exploration

The GLM over all five object exploration tasks revealed a significant effect of trial [TRIAL: $F(4,124)=12.85$, $P<.001$] and a significant interaction between trial and inanimate background [TRIAL × ENV: $F(4,124)=2.55$, $P<.05$]. Based on these effects, specific hypotheses were further analysed using planned contrasts.

3.1.3. Novel object exploration and habituation

When faced with two novel objects in the now-familiar open field (T_2), rats spent on average 30% of their total time on exploring the two objects. Neither social [SOC: $F(1,31)=0.01$, $P>.05$] nor inanimate background [ENV: $F(1,31)=0.02$, $P>.05$] had a significant effect on object exploration in T_2 (Fig. 3). When reexposed to the same situation (same objects in the same locations) in T_3, object exploration significantly decreased to about 23% of total time [$F(1,62)=12.92$, $P<.01$]. Again, however, no significant effect of social [SOC: $F(1,62)=0.97$, $P>.05$] or inanimate background [ENV: $F(1,62)=0.43$, $P>.05$] was detected on the change in object exploration from T_2 to T_3.

3.1.4. Recognition memory for spatial change and habituation

When one of the two objects was displaced in T_4, social background affected the change in total object exploration compared to T_3 [SOC: $F(1,62)=4.14$, $P<.05$], whereas inanimate background had no effect [ENV: $F(1,62)=0.28$, $P>.05$]. Thus, in isolation-reared rats (IE, IB), spatial novelty activated object exploration more than in group-reared rats (SE, SB), regardless of whether they had been reared in standard or enriched cages (Fig. 3). However, neither social [SOC: $F(1,62)=0.00$, $P>.05$] nor inanimate background [ENV: $F(1,62)=1.80$, $P>.05$] had a significant effect on the bias in exploration towards the spatially changed object, which was, however, rather weak and only short-lasting [INTERVAL × OBJECT: $F(3,204)=2.87$, $P<.05$] (Fig. 4). When reexposed to the same situation (same objects in the same locations) in T_5, the decrease in total object exploration compared to T_4 was more pronounced in enriched reared rats (IE, SE) than in rats reared

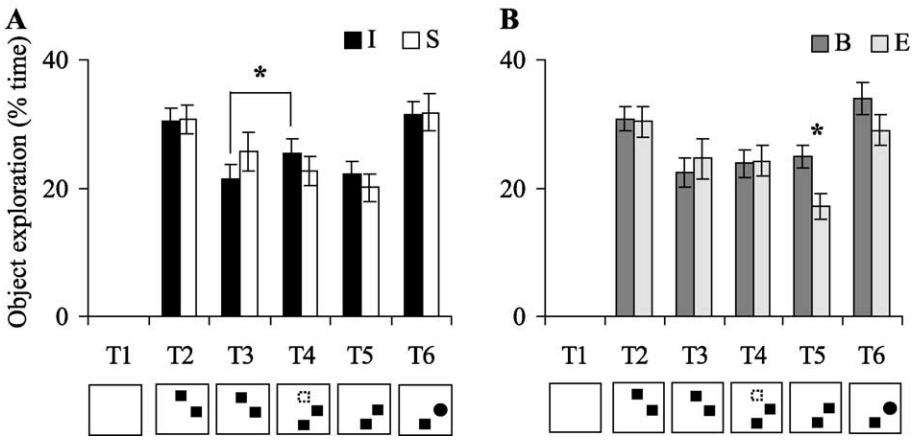

Fig. 3. Object exploration in percent of time (mean ± S.E.M.) in each of six consecutive exploratory trials in the open-field arena (T_1-T_6). (A) Isolation- (I = IB and IE pooled) versus group- (S = SB and SE pooled) reared rats. (B) Rats reared in barren (B = IB and SB pooled) versus enriched (E = IE and SE pooled) cages. * $P<.05$.

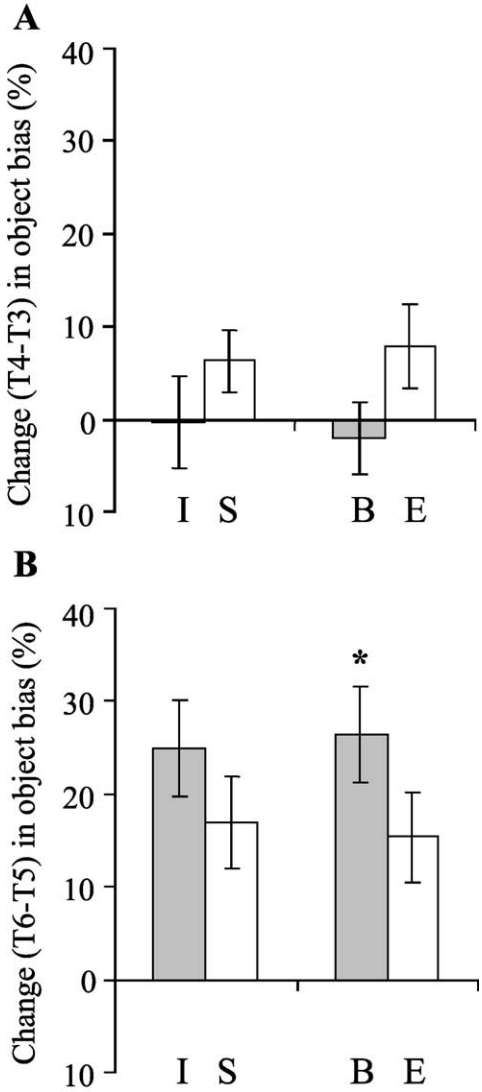

Fig. 4. Relative bias (mean ± S.E.M.) in exploration towards the displaced object in T_4 (A) and towards the new object in T_6 (B) compared to the previous trial (T_3 and T_5, respectively). I: Isolation-reared rats (IB and IE pooled); S: group-reared rats (SB and SE pooled); B: rats reared in barren cages (IB and SB pooled); E: rats reared in enriched cages (IE and SE pooled). * $P < .05$.

in barren cages [IB, SB; $F(1,62) = 7.43$, $P < .01$], regardless of whether they had been reared singly or in groups (Fig. 3).

3.1.5. Recognition memory for object novelty

Replacing one of the two objects by a novel object in T_6 resulted in a massive increase in total object exploration compared to T_5 [$F(1,62) = 32.83$, $P < .001$], and exploration was heavily biased towards the novel object [$F(1,62) = 48.09$, $P < .001$] (Fig. 3). Whereas neither social [SOC: $F(1,62) = 0.53$, $P > .05$] nor inanimate background [ENV: $F(1,62) = 0.64$, $P > .05$] had a significant effect on the increase in total object exploration, there was a significant interaction between inanimate background and bias in object exploration [ENV: $F(1,62) = 5.54$, $P < .05$]. Thus, regardless of social background, enriched reared rats showed less of a bias towards the novel object compared to rats reared in barren cages, which was due to enhanced habituation of object bias (Fig. 4).

3.2. L/D box

All subjects were placed in the light chamber, facing the hole in the wall that separated the light chamber from the dark chamber. Social background had a significant main effect on the latency to enter the dark chamber [SOC: $F(1,32) = 4.14$, $P < .05$]. Thus, isolation-reared rats (IE, IB) took significantly longer to enter the dark chamber for the first time (mean latency ± S.E.M.: 24.87 ± 2.67 s) compared to group-reared rats (SE, SB; mean latency ± S.E.M.: 18.78 ± 1.29 s), whereas inanimate background had no effect [ENV: $F(1,32) = 1.47$, $P > .05$] (Fig. 5). By contrast, reentering the light chamber after the first visit to the dark chamber was affected by inanimate background [ENV: $F(1,32) = 39.21$, $P < .001$], rather than social background [SOC: $F(1,32) = 1.15$, $P > .05$]. Thus, rats reared in barren cages (IB, SB) took significantly longer (mean latency ± S.E.M.: 34.48 ± 1.55 s) than rats reared in enriched cages (IE, SE; mean latency ± S.E.M.: 18.56 ± 2.01 s), regardless of whether they had been reared singly or in groups (Fig. 5).

All rats repeatedly moved back and forth between the two chambers. However, although there was no significant effect of social [SOC: $F(1,32) = 0.67$, $P > .05$] or inanimate background [ENV: $F(1,32) = 0.46$, $P > .05$] on the total number of crossings between the two chambers, there was a significant interaction between time and inanimate background [INTERVAL × ENV: $F(3,96) = 16.27$, $P < .001$]. Thus, whereas in the first timebin the rate of crossings was significantly higher in rats reared in enriched cages (IE, SE) compared to rats reared in barren cages (IB, SB) [INTERVAL1: $F(1,32) = 15.60$, $P < .01$], it rapidly habituated in enriched reared rats and to a much greater extent than in barren-reared rats, such that in Timebins 3 and 4, enriched reared rats moved back and forth significantly less often than standard reared rats [INTERVAL3: $F(1,32) = 8.43$, $P < .01$; INTERVAL4: $F(1,32) = 4.40$, $P < .05$]. By contrast, social background had no significant effect on the change in the rate of crossings between the light and dark chamber [INTERVAL × SOC: $F(3,96) = 1.33$, $P > .05$] (Fig. 5).

Together with the decrease in the rate of crossings between the two chambers, time spent in the dark chamber compared to the light chamber increased from about 60% of time in the first timebin to about 80% of time in Timebins 3 and 4 [INTERVAL: $F(3,96) = 43.69$, $P < .001$]. However, rats reared singly in barren cages (IB) displayed a pattern of time spent in either chamber that was distinctively different from rats of all other treatment groups. Thus, they spent more than 50% more time in the light chamber than all other rats as shown by a significant interaction between social and inanimate background with respect to total time in

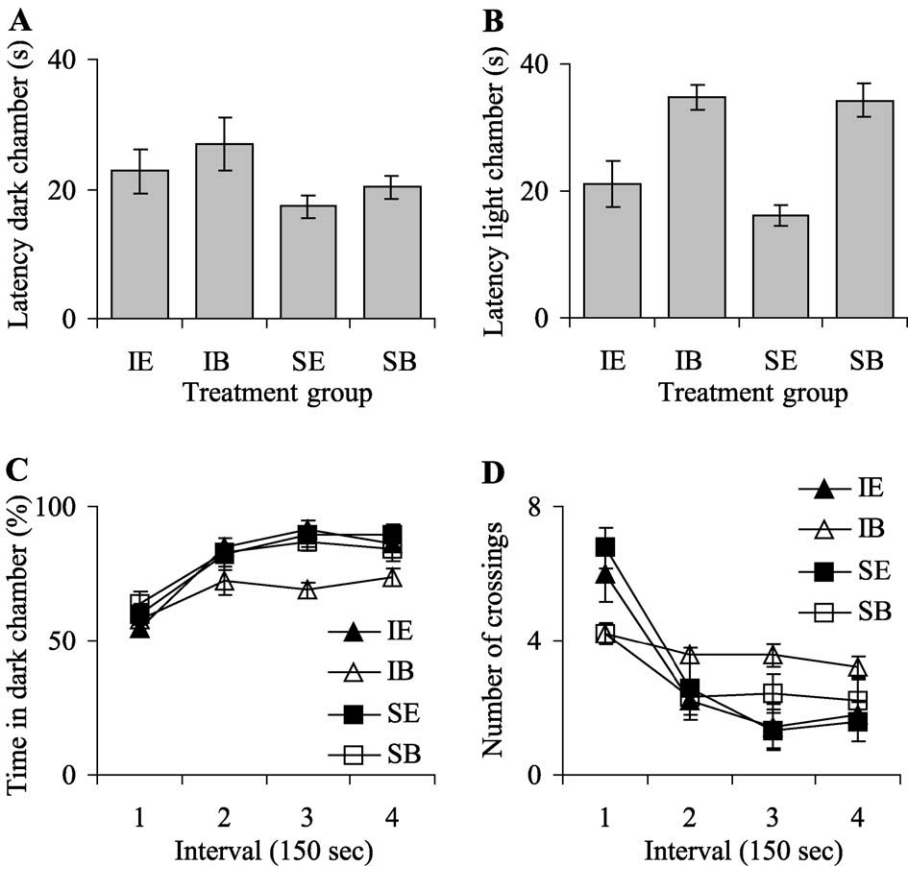

Fig. 5. Results of the L/D box test. (A) Latency to enter the dark chamber. (B) Latency to return to the light chamber. (C) Total time in percent spent in the dark chamber across four intervals of 150 s each. (D) Number of crossings between light and dark chambers across the four intervals. IB: Rats reared singly in barren cages; IE: rats reared singly in enriched cages; SB: rats reared in groups in barren cages; SE: rats reared in groups in enriched cages. Figures are based on group mean ± S.E.M.

the light chamber [SOC × ENV: $F(1,32) = 4.16$, $P < .05$]. This difference was due to a dramatically reduced rate of habituation in IB rats (Fig. 5). However, due to the slightly intermediate performance of SB rats, the GLM only revealed a signifcant time by inanimate background interaction [INTERVAL × ENV: $F(3,96) = 3.79$, $P < .05$], whereas the interaction between social and inanimate background with time failed to reach significance [INTERVAL × ENV × SOC: $F(3,96) = 1.30$, $P > .05$]. Thus, whereas time in the light chamber did not differ between groups in the first timebin [INTERVAL1: $F(1,32) = 1.04$, $P > .05$], rats of all other treatment groups showed a clear preference for the dark chamber as from Timebin 2 onwards, whereas in IB rats, the preference for the dark chamber developed at a much slower rate, as shown by a significant difference compared to the other treatment groups in Timebins 3 and 4 [INTERVAL3: $F(1,32) = 11.87$, $P < .01$; INTERVAL4: $F(1,32) = 4.72$, $P < .05$]. Although there was no significant interaction between social and inanimate background with respect to the rate of habituation in the number of crossings between the two chambers [INTERVAL × SOC × ENV: $F(3,96) = 0.20$, $P > .05$], part of the difference in habituation to the light chamber in IB rats might be explained by a reduced rate of habituation in the number of crossings between the two chambers, which persisted on a higher level in IB rats than in SB rats (Fig. 5).

3.3. Morris water maze

3.3.1. Spatial training

All rats reliably learned to locate the hidden platform within the 6 days of training in the Morris water maze. Escape latency decreased from a daily mean of 53.93 ± 2.89 s on Day 1 to 7.27 ± 0.38 s on Day 6 [DAY: $F(5,160) = 131.73$, $P < .001$] (Fig. 6). As swim speed did not markedly change across days, there was a similar decrease in average path length to the hidden platform [DAY: $F(5,160) = 157.87$, $P < .001$]. However, there was a significant effect of inanimate background on spatial learning with respect to both escape latency [ENV: $F(1,32) = 6.98$, $P < .01$] and path length [ENV: $F(1,32) = 5.55$, $P < .05$]. This was due to a faster rate of acquisition of this spatial task in rats reared in enriched cages (IE, SE) compared to barren-reared rats (IB, SB), as shown by a significant interaction between inanimate background and

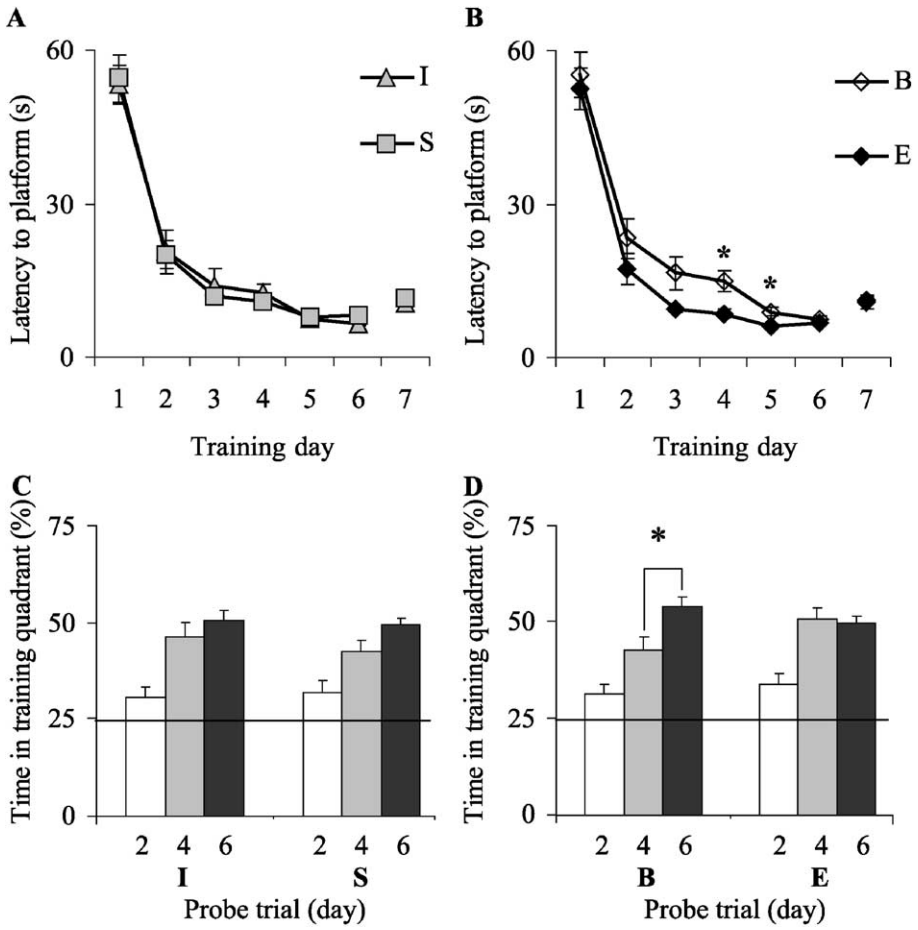

Fig. 6. Results of the Morris water maze test. Upper panels: Latency in seconds (mean ± S.E.M.) to locate the hidden platform across the 6 days of spatial training, and to locate the visually cued platform on Day 7. (A) Isolation- (I = IB and IE pooled) versus group- (S = SB and SE pooled) reared rats. (B) Rats reared in barren (B = IB and SB pooled) versus enriched (E = IE and SE pooled) cages. Lower panels: Time in percent (mean ± S.E.M.) spent in the training quadrant in probe trials on Days 2, 4 and 6. (C) Isolation- (I) versus group- (S) reared rats. (D) Rats reared in barren (B) versus enriched (E) cages. * $P < .05$.

day of training [DAY × ENV: $F(5,160) = 2.23$, $P < .05$]. Thus, although rats reared in enriched cages did not differ from barren-reared rats on Day 1, a difference emerged on Days 2 and 3 and became significant on Days 4 [escape latency: $F(1,32) = 10.41$, $P < .01$; path length: $F(1,32) = 10.20$, $P < .01$] and 5 [escape latency: $F(1,32) = 6.94$, $P < .01$; path length: $F(1,32) = 6.60$, $P < .05$] (Fig. 6). Importantly, enriched reared rats had reached asymptotic levels of performance as early as on Day 3, whereas standard reared rats needed the full 6 days of training to meet a similar performance level with respect to both escape latency [DAY6: $F(1,32) = 1.47$, $P > .05$] and path length [DAY6: $F(1,32) = 1.17$, $P > .05$]. Furthermore, the differences between enriched and standard reared rats were mainly due to differences in performance in the first daily trials [escape latency: DAY × ENV: $F(5,160) = 2.48$, $P < .05$; path length: DAY × ENV: $F(5,160) = 4.12$, $P < .01$], whereas there were no significant differences in the fourth daily trials [escape latency: DAY × ENV: $F(5,160) = 0.19$, $P > .05$; path length: DAY × ENV: $F(5,160) = 0.20$, $P > .05$]. By contrast, social background had no effect on performance in the acquisition of this spatial task [escape latency: $F(1,32) = 0.28$, $P > .05$; path length: $F(1,32) = 0.05$, $P > .05$] (Fig. 6).

3.3.2. Probe test

On days when rats were subjected to probe tests of 60 s without a platform in the pool prior to being subjected to the regular training trials, time spent in the training quadrant increased from an average of 31.96 ± 1.92% on Day 2 over 45.78 ± 2.33% on Day 4 to 50.73 ± 1.60% on Day 6. Thus, in parallel to the improvement seen in the training trials, preference for the training quadrant increased across days [PROBE: $F(2,64) = 34.232$, $P < .01$]. Moreover, there was a significant interaction between inanimate background and day of probe test]PROBE × ENV: $F(2,64) = 3.29$, $P < .05$], indicating that enriched reared rats (IE, SE) more rapidly acquired a strong preference for the training quadrant compared to barren-reared rats (IB, SB), although the difference on Day 4 failed to reach significance [PROBE4: $F(1,32) = 3.06$, $P = .09$] (Fig. 6).

3.3.3. Visual cue training

On the day following the 6 days of spatial training, rats were subjected to four trials with a visually cued platform, position of which was varied from trial to trial. However, neither social [escape latency: $F(1,32)=0.58$, $P>.05$; path length: $F(1,32)=0.26$, $P>.05$] nor inanimate background [escape latency: $F(1,32)=0.14$, $P>.05$; path length: $F(1,32)=0.15$, $P>.05$] had an effect on performance in this visual navigation task (Fig. 6).

3.4. Restraint stress

3.4.1. Basal values

Basal levels of plasma ACTH (119.12 ± 3.34 pg/ml) and plasma corticosterone (71.06 ± 5.55 ng/ml) were very consistent among the whole population of rats, and neither social [ACTH: SOC: $F(1,32)=0.41$, $P>.05$; corticosterone: SOC: $F(1,32)=0.02$, $P>.05$] nor inanimate background [ACTH: ENV: $F(1,32)=0.07$, $P>.05$; corticosterone: ENV: $F(1,32)=1.34$, $P>.05$] had a significant effect (Fig. 7).

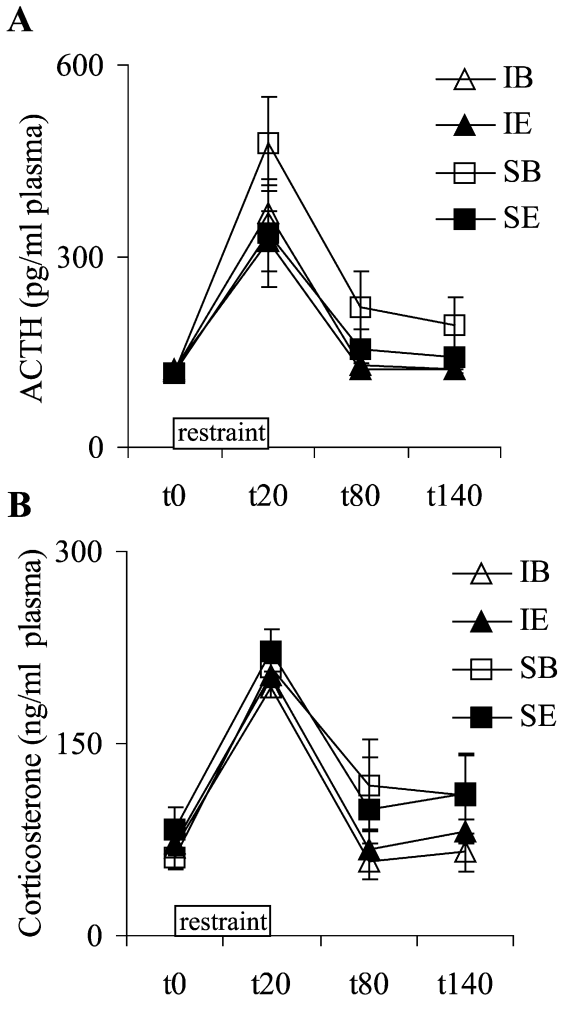

Fig. 7. Basal levels (t_0), peak response (t_{20}) and recovery (t_{80}, t_{140}) of plasma ACTH (A) and plasma corticosterone (B) levels in response to 20-min restraint. IB: Rats reared singly in barren cages; IE: rats reared singly in enriched cages; SB: rats reared in groups in barren cages; SE: rats reared in groups in enriched cages.

3.4.2. Acute stress response and recovery

Exposure to 20-min restraint induced a threefold increase in both plasma ACTH (375.74 ± 33.05 pg/ml) and plasma corticosterone levels (207.19 ± 5.80 ng/ml; Fig. 7). Sixty minutes after termination of restraint-induced stress, both ACTH (156.15 ± 17.16 pg/ml plasma) and corticosterone levels (85.23 ± 14.32 ng/ml plasma) were almost back to basal levels and did not substantially change during the subsequent 60 min (ACTH: 144.24 ± 12.64 pg/ml plasma; corticosterone: 91.55 ± 11.98 ng/ml plasma). All measures were surprisingly consistent throughout the population, except for four rats, each from a different social group that showed extreme values both in terms of the acute stress response and during recovery (Fig. 7).

Although neither social nor inanimate background had a significant effect on response measures and recovery, enriched reared rats tended to show a slightly attenuated ACTH peak response compared to rats reared in barren cages [ACTH: SOC: $F(1,32)=0.35$, $P>.05$; ENV: $F(1,32)=3.63$, $P=.07$; corticosterone: SOC: $F(1,32)=2.32$, $P>.05$; ENV: $F(1,32)=0.88$, $P>.05$], whereas recovery to basal levels of both ACTH and corticosterone tended to be retarded in socially reared rats compared to isolation-reared rats [ACTH: SOC: $F(1,31)=3.41$, $P=.07$; ENV: $F(1,31)=1.42$, $P>.05$; corticosterone: SOC: $F(1,31)=2.74$, $P=.11$; ENV: $F(1,31)=0.01$, $P>.05$].

4. Discussion

In this study, social (isolation versus group rearing) and inanimate stimulations (barren cages versus enriched cages) were varied independently to examine the effects of isolation rearing and environmental enrichment on the behavioural responses of adult rats in several standard behavioural tests and on their endocrine responses to a standard stressor. The results show that isolation rearing and environmental enrichment produce dissociable effects on behaviour that are consistent across variation in environmental background. Thus, whereas isolation rearing, regardless of inanimate background, primarily enhanced activity in response to several situations of novelty, environmental enrichment, regardless of social background, mainly accelerated habituation to novelty and improved spatial learning and memory. However, the study also revealed significant interactions between social and inanimate stimulation, resulting in effects that were specific to a particular environmental background. Thus, in the L/D box, isolates reared in barren cages exhibited a pattern of activity that was distinctively different from the three other treatment groups. The significance of these effects and their implications for animal experimentation are discussed below.

4.1. Locomotor activity and object exploration in the open field

Isolation-reared rats were more active in a novel open field compared to group-reared rats (T_1). Spontaneous hyperactivity in response to a novel environment is perhaps the most consistent effect of isolation rearing in rats (Robbins et al., 1996; Hall, 1998; Weiss, 2001), except for the Sprague–Dawley strain (Geyer at al., 1993; Weiss et al., 2000). In the present study, spontaneous hyperactivity occurred irrespective of inanimate background, indicating that it might represent a robust effect induced by deprivation of early social stimulation, rather than deprivation of environmental stimulation in general. This is consistent with a recent study reporting spontaneous hyperactivity in isolation-reared male Wistar rats, regardless of whether they were housed on sawdust or grid floor (Weiss et al., 1999). The robustness of this effect might also explain why it has been observed across many laboratories, in spite of the usual differences among laboratories in housing conditions, management, test apparatus and test protocol.

Isolation rearing further enhanced overall object exploration in response to spatial displacement of one of two familiar objects in T_4. It has been suggested that isolation-reared rats are generally more reactive to novelty (Robbins et al., 1996; Hall, 1998), but behavioural expression seems to depend highly on context (Hall et al., 1997, 2000). In line with this, isolates in the present study did not exhibit enhanced responses under all conditions of novelty. Thus, isolation rearing had no effect on activity and object exploration when two novel objects were introduced in T_2 and when one of the two was replaced by a different object in T_6. One possible explanation for this could be that spatial novelty, rather than novelty per se, might underlie isolation-induced hyperactivity in novel environments. Alternatively, the lack of effect of isolation rearing on object exploration in T_2 and T_6 might be due to ceiling effects, since object exploration reached maximal levels in all treatment groups under these two conditions. Furthermore, it has been previously suggested that behavioural competition between increased exploratory tendencies and general hyperactivity might lead to conflicting findings depending on the exact test situation (Einon and Morgan, 1976; Hall, 1998). Thus, in order to reach unambiguous conclusions, the effect of isolation rearing on responses to object novelty might need to be studied using an approach that is not confounded with overall activity levels (cf. Sahakian et al., 1977).

In contrast, environmental enrichment accelerated habituation of locomotor activity both within and between trials as well as habituation of object exploration, although the latter was significant only in T_5. These results confirm recent findings demonstrating enhanced habituation in exploratory activity to novel objects in a familiar environment (Zimmermann et al., 2001). However, in contrast to an earlier study (Tees, 1999), environmental enrichment did not result in enhanced object exploration following spatial or object change. On the contrary, although enriched reared rats did not exhibit reduced object exploration in Trials 4 (spatial change) and 6 (object change), they habituated faster to spatial change as indicated by reduced exploration in Trial 5. Furthermore, the bias in exploration towards the replaced object (T_6) habituated faster in enriched reared rats, resulting in a smaller overall bias towards the replaced object. This might have important implications for the interpretation of exploratory behaviour following spatial or object change in terms of recognition memory. Thus, enhanced exploration following spatial and object change and a stronger bias towards the changed object are generally interpreted in terms of better recognition memory (e.g., Buhot and Naïli, 1995). However, the validity of this interpretation might critically depend on the length of time over which exploration is measured, because faster habituation to novelty induced by spatial or object change might lead one to misinterpret lower total exploration scores in terms of poorer recognition memory. Nevertheless, as the initial exploration scores following both spatial and object change were neither affected by social nor inanimate background, there is no evidence to suggest that either of these manipulations affected short-term recognition memory for location and identity of previously explored objects.

4.2. Activity in the L/D box

In the L/D box, isolation rearing enhanced the latency to enter the dark chamber from the light chamber in which the rats were placed at the start of the test. A longer latency to enter the dark chamber is generally considered to reflect reduced anxiety or fearfulness (but see Chaouloff et al., 1997). Thus, the present results might suggest that isolation rearing reduces anxiety. This interpretation contrasts with many other studies in which isolation-reared rats were generally found to be more anxious or fearful than group-reared controls (Robbins et al., 1996; Hall, 1998). However, the anxiogenic profile of isolation-reared rats is also characterised by their enhanced resistance to enter a novel environment (e.g., Gentsch et al., 1982; Einon and Tye, 1975; Zimmermann et al., 2001). Thus, when rats were released in the light chamber of the L/D box, aversion towards the light chamber might have competed with resistance to enter the dark, but novel, chamber over expression in terms of two conflicting behaviours. Thus, interpreting a longer latency to enter the dark chamber in terms of reduced anxiety or fearfulness appears equivocal, especially in isolation-reared rats.

Conversely, enriched reared rats took considerably less time to reenter the light chamber after their first visit to the dark chamber compared to rats reared in barren cages. The shorter exploration of the dark chamber on their first visit is in line with the general picture of enhanced habituation to novelty in enriched reared rats, as also seen in the open-field and object exploration trials discussed above. Also in line with this, enriched reared rats initially moved back and forth

between the light and dark chambers at a much higher rate which, however, habituated much faster and to a much greater extent, compared to rats reared in barren cages. Thus, although a lower number of crossings between the two chambers are generally considered to reflect increased anxiety (Chaouloff et al., 1997), the present results appear to favour an interpretation of this measure in terms of exploratory activity rather than anxiety. A possible explanation for this might be that the rats had previously been subjected to a series of exploratory tasks in an open field, which might have attenuated their anxiety levels in the L/D box. Although exploration is clearly affected by anxiety, exploratory activity and emotional reactivity are considered to represent independent dimensions rather than the two extremes of a unitary variable (Berton et al., 1997; Denenberg, 1969; Hall et al., 2000; Jahkel et al., 2000). Thus, when levels of anxiety are reduced (or when a normally aversive environment is made less aversive), the control over behaviour might be shifted from emotional reactivity towards exploratory activity.

The ambiguous nature of the L/D box test might also explain the strong interaction between social and inanimate background, resulting in an idiosyncratic behavioural profile in isolates that were reared in barren cages. Whereas isolation rearing induces hyperactivity in response to a novel and aversive environment (Robbins et al., 1996; Hall, 1998; Weiss, 2001), barren housing retards habituation of exploratory activity (Zimmermann et al., 2001). Thus, a combination of hyperactivity and retarded habituation in isolates reared in barren cages might explain their persistent activity in the L/D box. This raises the possibility that some of the symptoms currently subsumed under the heading 'isolation syndrome' (Robbins et al., 1996) might in fact depend on environmental factors other than, or in addition to, early social deprivation. However, this is not to say that isolation rearing in barren environments and group rearing in enriched environments represent extremes of a single variable in terms of environmental stimulation as suggested by others (e.g., Van Praag et al., 2000). On the contrary, the present result favours the view that the resulting phenotype might depend on selective effects of social and inanimate stimulation on dissociable brain functions. Further studies in which the novelty and aversiveness of the test environment are varied independently might further elucidate these relationships.

4.3. Spatial learning and memory in the Morris water maze

Improved spatial learning and memory is one of the most consistent findings in the literature on environmental enrichment (Van Praag et al., 2000). The present results confirm this finding and extend its validity by showing that it generalizes across variation in social background. Thus, enriched reared rats showed better spatial learning than rats reared in barren cages, irrespective of whether they had been reared singly or in groups. This effect is unlikely to be due to noncognitive factors (Wolfer and Lipp, 2000): thigmotaxis and passive floating were observed only on the first training day when rats were unfamiliar with the task, and there was no difference between treatment groups in the ability to navigate the platform when it was visually cued.

Environmental enrichment is, however, not a precondition for the animals to learn the spatial navigation task. After 6 days of training, performance of rats reared in barren conditions reached the same level of performance in terms of time and accuracy to navigate the hidden platform. However, environmental enrichment appears to enhance the speed at which rats acquire spatial knowledge. Thus, whereas enriched reared rats reached asymptotic performance on Day 3, rats reared in barren cages needed 5–6 days to reach the same level of performance. This was also reflected by the fact that in enriched reared rats, probe trial performance reached asymptotic levels on Day 4, whereas in rats reared in barren cages, it improved further from Days 4 to 6. Furthermore, the difference in performance between enriched and barren-reared rats was mainly due to the difference in the first daily trial, whereas no differences were found in subsequent daily trials. This suggests that it is the transformation of spatial knowledge from short-term memory into long-term memory that is improved by environmental enrichment.

4.4. Restraint-induced stress

Stress is a major intervening variable in many behavioural tests and is also known to interfere with learning and memory, although in some situations, corticosteroids that are massively released under conditions of stress appear to facilitate memory consolidation (De Kloet et al., 1999). It is, therefore, important to assess whether the behavioural effects of isolation rearing and environmental enrichment reported here can be explained in terms of differences in the responses to external stressors. Present evidence is rather controversial. Several authors have reported that isolation-reared rats have enhanced responses to stressors (e.g., Sahakian et al., 1977; Gamallo et al., 1986; Heritch et al., 1990; Plaznik et al., 1993; Weiss, 2001), and it was suggested that their anxiogenic behavioural profile might be related to this. However, whereas Gentsch et al. (1981) found smaller increases in corticosterone levels in isolates compared to social controls after exposure to an open field (though this could have been due to an acute isolation experience in the socials; cf. Hall, 1998), Hall et al. (2001) found no effect of isolation rearing on corticosterone after exposure to a forced swim test, and Weiss (2001) found increased basal and response levels of ACTH, and slightly increased corticosterone levels, after exposure to a startle session. This variation suggests that the much more consistently reported anxiogenic behavioural profile of isolates might be independent of their sensitivity to stressors (reviewed by Hall, 1998).

The present results show that basal levels of both ACTH and corticosterone were unaffected by environmental back-

ground. However, whereas enriched reared rats tended to show a slightly attenuated ACTH peak response, recovery to basal levels of both ACTH and corticosterone tended to be retarded by social rearing. Retarded recovery in socially reared rats was entirely due to four individuals, each from a different social group. As no such outliers were detected in the five other groups, they are unlikely to represent a particular social position within groups of three males (e.g., dominants, subdominants, subordinates). More likely, they might have been members of socially unstable groups. When Hurst et al. (1999) looked at the effect of group size in standard laboratory cages on various behavioural, physiological and morphological changes, they found that stocking density was largely uncorrelated with all of these parameters, whereas there were marked differences between replicate groups of similar stocking density that could be predicted by behavioural indicators of social stability within groups. This raises the possibility that the social dynamics between animals within groups might be an important factor to take into account in animal experiments (Würbel, 2002). Unfortunately, from the present study, no data are available on home cage behaviour of the rats to examine this possibility further. Alternatively, the four outliers might have been caused by sampling or analysis errors. However, post hoc analysis revealed no significant effects on the outcome of the behavioural tests discussed above when these rats were excluded (data not shown). Whether or not the reduced ACTH peak response in enriched reared rats might account for behavioural differences in this study remains unclear. Given the small effect size and the fact that no effect was detected on the downstream corticosterone response, it is, however, unlikely that this subtle difference had a significant effect on the outcome of the behavioural tests.

These results clearly contrast with those of Weiss (2001), while confirming and extending those of Hall et al. (2001) by showing that the lack of effect of isolation rearing on basal and response measures of HPA axis functioning was consistent across variation in inanimate background. Social isolation has earlier been suggested to constitute a stress treatment (e.g., Holson et al., 1991), but often social isolation is confounded with other differences in husbandry procedures that might be more stressful for isolates than for group-reared controls. For example, isolates tend to respond more emotionally—and sometimes aggressively—to handling and are, therefore, often picked up by their tail when changing cages (Weiss, personal communication), which is known to be aversive to rats. This raises the possibility that differences in stress responses might partly depend on differences in husbandry procedures, especially in terms of human–animal interactions.

5. Conclusions

The present results confirm and extend the findings of previous studies reporting enhanced activity in isolation-reared rats in response to novel environments (Robbins et al., 1996; Hall, 1998) by showing that isolation rearing, regardless of inanimate background, enhanced activity in response to environmental novelty across several situations (novel open field, spatial change of objects, L/D box). However, they provide no evidence for enhanced reactivity or retarded habituation to novelty in general (e.g., novel objects). Furthermore, it remains unclear whether increased anxiety is a general characteristic of isolation-reared rats and whether this might explain their enhanced activity in response to environmental novelty. The present results on activity in the L/D box appear inconclusive, as patterns of activity suggest that the usual measures of anxiety (e.g., number of crossings, time in light chamber) were strongly confounded with exploratory activity. Because most unconditioned tests of anxiety involve spatial novelty, they do not allow for a discrimination between novelty exploration and anxiety (Hall, 1998). However, systematic variation of the novelty and aversiveness of the test situation in combination with the four environmental treatments used here might allow to obtain a clearer picture. Nonetheless, isolation rearing does not appear to induce enhanced responses to stressors, and the behavioural effects reported here were uncorrelated with HPA reactivity.

With respect to environmental enrichment, the present findings confirm and extend previous findings showing enhanced habituation of exploratory activity in response to novelty (Zimmermann et al., 2001) and improved spatial learning and memory (Van Praag et al., 2000). These effects are independent of social background and are, therefore, likely to represent robust effects that depend on inanimate stimulation.

Taken together, this study shows that systematic variation of both social and inanimate background might help distinguish between robust results that generalise across environmental conditions and results that are idiosyncratic to particular environmental conditions (Würbel, 2002). This is important in view of validating behavioural symptoms induced by environmental (or other experimental) manipulations in terms of animal models of symptoms associated with human psychiatric conditions. Moreover, because of the eminent biological significance of both social and inanimate stimulation during early ontogeny, systematic variation of these two factors might also reveal biologically relevant interactions between a particular environmental (or other experimental) manipulation and the environmental background of the animals. Thus, the idiosyncratic profile in the L/D box of isolates reared in barren cages might reflect an interaction between the effects of isolation rearing on reactivity to spatial novelty and of environmental enrichment on habituation of exploratory activity to novelty. Identifying such interactions might help to refine animal models that are based on environmental manipulations by improving both the face validity of the elicited behavioural symptoms and the construct validity of the hypothesised mechanisms underlying their expression.

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