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Early-life handling stimulation and environmental enrichment Are some of their effects mediated by similar neural mechanisms?

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Abstract

Neonatal (early) handling (EH) and environmental enrichment (EE) of laboratory rodents have been the two most commonly used methods of providing supplementary environmental stimulation in order to study behavioral and neurobiological plasticity. A large body of research has been generated since the 1950s, unequivocally showing that both treatments induce profound and long-lasting behavioral and neural consequences while also inducing plastic brain effects and being "protective" against some age-related deficits. The present work is aimed at reviewing the main neurobehavioral effects of both manipulations, with the final purpose of comparing them and trying to find out to what extent the effects of both treatments may share (or not) possible neural mechanisms. © 2002 Published by Elsevier Science Inc.

Keywords: Early experience; Neonatal handling; Environmental enrichment; Stress; Anxiety; Learning; Memory; Neural plasticity; Hippocampus; Age-related deficits

1. Introduction

Early postnatal stimulation [early (neonatal) handling (EH), in its most frequent form] and environmental enrichment (EE, the exposure of juvenile/adult animals, for weeks or months, to environments rich in sensory stimulation) have been shown to produce profound and long-lasting behavioral and neurobiological effects. Both treatments have been reported to variously influence activity, exploration, emotionality/fearfulness and unconditioned and conditioned learning tasks in laboratory rodents. In addition, both manipulations appear to induce "protective" effects against age-related cognitive deficits and some associated physiological and neural processes (e.g. Anisman et al., 1998; Fernández-Teruel et al., 1997; Mohammed et al., 1993; Renner and Rosenzweig, 1987).

However, although some of the recently reported effects of both treatments may be seen as rather similar (e.g. long-term improvements in spatial learning and "protective" effects against age-related behavioral deficits) (e.g. Fernández-Teruel et al., 1997; Meaney et al., 1988; Pham et al., 1997; Venable et al., 1988), the research conducted on psychobiological and neurobiological effects of both manipulations have traditionally followed different paths, i.e. since the 1950–1960s researchers interested in the effects of neonatal handling have focused mainly on its emotional/endocrine effects (e.g. Levine and Broadhurst, 1963; Levine, 1957; Levine et al., 1967), while research on EE was essentially focused on its morphological consequences in the central nervous system and associated changes in exploration or problem-solving tasks (e.g. Denenberg and Morton, 1962a,b; Hebb, 1949; Hymovitch, 1952; see Renner and Rosenzweig, 1987 for a review).

The use of experimental designs to simultaneously compare the effects and/or possible mechanisms of neonatal stimulation (through handling) and EE have been, thus far, very scarce. Thus, it is our purpose here to review the main effects and/or putative mechanisms of both manipulations, with the objective of presenting similarities and differences between them and some possible common pathways through which both treatments appear to produce at least some of their effects. To this aim, special consideration will be devoted to research in which both treatments have been

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administered separately and combined in factorial experimental designs.

2. Effects of EH and EE on spontaneous behavior, fearfulness and stress-related hormonal responses

2.1. Early handling

Levine et al. (Levine, 1956; Levine et al., 1956) provided the first demonstration that EH induced an improvement in the ability of rats to learn a two-way active avoidance task. Several other studies have since confirmed those results, additionally showing that the improving effects of EH extend to several different strains/lines of rats and are remarkably long-lasting (Denenberg and Karas, 1961; Escorihuela et al., 1991, 1992, 1994a, 1995a,b; Levine and Wetzel, 1963; Núñez et al., 1995). As there is substantial evidence indicating that the initial acquisition of two-way active avoidance is anxiety-mediated (with relatively high levels of anxiety/emotionality resulting in impaired acquisition) (e.g. Aguilar et al., 2002a; Boix et al., 1988, 1992; Brush, 1991; Fernández-Teruel et al., 1991b; Gray and Lalljee, 1974; Weiss et al., 1968), it has been proposed that EH effects on that test are mainly due to an enduring reduction of fearfulness.

Additional support for this proposal arises from a wide range of studies most often demonstrating that EH increases activity and specific exploratory behavior, usually associated with a decrease in defecation, in a variety of unconditioned laboratory tests involving different degrees of novelty, such as the open-field test (e.g. Denenberg et al., 1978; Fernández-Teruel et al., 1992a; Levine and Broadhurst, 1963; Levine et al., 1967), "timidity" tests and exposure to "novel/unknown" cages (Ader, 1959, 1968; Eells, 1961; Ferré et al., 1995; Núñez et al., 1995) (see also Table 1), the hole-board test (Fernández-Teruel et al., 1992a; File, 1978), tests of "tactual variation seeking" (De Nelsky and Denenberg, 1967) and tunnel labyrinths (not involving deprivation) (Fernández-Teruel et al., 1991a, 1992b, 1993). Moreover, when tests more specifically measuring anxiety or fearfulness (either unconditioned or conditioned) have been used, it has been shown that EH treatment increases exploration of the open arms in the elevated plus-maze (Fernández-Teruel et al., 1990; McIntosh et al., 1999; Núñez et al., 1995) and the number of visits to, and time spent, in open/illuminated compartments in a tunnel labyrinth and in the dark–light box (Fernández-Teruel et al., 1991b, 1992b; Steimer et al., 1998) (see also Table 1), it decreases hyponeophagia (i.e. novelty-induced fear) (Bodnoff et al., 1987; Steimer et al., 1998), while it also reduces suppression of drinking and freezing in the lick-suppression conflict test (Núñez et al., 1996). Thus, taking into account the abovementioned effects of EH on two-way avoidance acquisition and on tests measuring spontaneous or conditioned emotionality/fearfulness and the observation that it also reduces learned helplessness in rats (Tejedor-Real et al., 1998), it seems safe to conclude that, from a behavioral standpoint, EH-exposed subjects appear to have an improved ability to adapt or to efficiently cope with highly challenging and stressful environmental conditions.

A large body of research dealing with the influences of infantile stimulation on endocrine function have substantiated and completed the picture drawn from those behavioral studies. Thus, EH has been reported to lead to an earlier maturation of the adrenocortical response, reduced corticosterone, ACTH and prolactin secretion in response to several (either unconditioned or conditioned) stressors and a faster return to basal pre-stress hormonal levels than nonhandling (e.g. Ader and Grota, 1969; Levine, 1957; Levine et al., 1957; Meaney et al., 1991; Núñez et al., 1996; Steimer et al., 1998). It is especially worth noting that those EH effects on endocrine responses to stress (as well as on behavior) are extremely long-lasting (probably the whole life span) and appear to be more marked in strains/lines of rats or mice that are relatively more sensitive to stress (e.g. Anisman et al., 1998; Hennessy et al., 1982; Meaney et al., 1988, 1991; Steimer et al., 1998; Treiman et al., 1970) (see also Table 2).

2.2. Environmental enrichment

As noted by Rosenzweig (1979) (see also Renner and Rosenzweig, 1987), M.V. Malacarne (1744–1816) was the first author who investigated the influence of EE treatment on the central nervous system. He found that birds that received enriched training had larger brains (especially evident in the cerebellum) than their nonenriched and isolated counterparts coming from the same clutch of eggs (see Renner and Rosenzweig, 1987). However, systematic experimental research on the influence of rearing in enriched environments (large cages, with several types of objects and spatial configurations, which are frequently changed) upon behavior (and thereafter upon brain development) can be considered to have started in 1947, when D.O. Hebb reported that animals raised in an EE showed superior learning ability than their nonenriched counterparts (Hebb, 1949; Forgays and Forgays, 1952).

A considerable amount of literature has since been accumulated on EE effects upon spontaneous behavior, learning and neurobiological processes (e.g. Fernández-Teruel et al., 1997; Mohammed et al., 1993; Renner and Rosenzweig, 1987; Van Praag et al., 2000). To summarize, a frequent effect of EE treatment on spontaneous activity/exploration in novelty situations is a long-lasting increase in exploratory activity, for instance in the open-field test (Denenberg et al., 1978; Ferchmin and Eterovic 1980; Larsson et al., this issue; Ray and Hochhauser, 1969; Widman and Rosellini, 1990) and in the hole-board test (Escorihuela et al., 1994a,b; Fernández-Teruel et al., 1992a and this issue), although there are also studies reporting contradictory results (e.g. Fernández-Teruel et al., 1992a; Freeman

Table 1
The subjects used for the experiments shown in this table (as well as for those of Table 4) were Roman high- (RHA-I/Verh) and low-avoidance (RLA-I/Verh) rats

	RHA-I/Verh		RLA-I/Verh	
	Nonhandled	Handled	Nonhandled	Handled
(A) Timidity				
Number of litters	11	11	7	7
Approach to the frontal wall (%)	51	93*	0*	61**
Exploration of a novel object (%)	35	66*	0*	58**
(B) Dark–light hexagonal tunnel maze				
Number of animals	12	8	12	8
Day 1				
Total activity (counts)	191.7 ± 9.4	234.6 ± 19.9***	132.2 ± 5.4***	147.1 ± 7.0
Latency to enter to the center (s)	93.8 ± 12.3	36.0 ± 14.8****	147.4 ± 18.7	119.7 ± 38.8
Entries to the lit center	14.2 ± 2.7	20.0 ± 3.3	3.2 ± 0.7***	6.4 ± 2.2$^{\#}$
Day 2				
Total activity (counts)	238.3 ± 9.2	273.7 ± 17.3	108.8 ± 11.2***	141.9 ± 17.7$^{\#}$
Latency to enter to the center (s)	63.5 ± 14.6	15.0 ± 3.7****	304.0 ± 34.3***	162.4 ± 58.1†
Entries to the lit center	13.7 ± 3.4	29.1 ± 5.6***	0.5 ± 0.3***	6.9 ± 2.5†
(C) Dark–light box				
Number of animals	22	8	22	8
Entries to the lit compartment	5.3 ± 0.9	9.0 ± 1.4‡	2.5 ± 0.4	4.0 ± 0.8
Time in the lit compartment (s)	54.9 ± 10.2	105.4 ± 16.3‡	43.7 ± 10.0	79.5 ± 21.0

These inbred strains have been derived (starting in 1993) through brother/sister mating from the outbred RHA/Verh and RLA/Verh lines, which have been selected and bred since 1972 for their rapid (RHA/Verh) vs. extremely poor (RLA/Verh) ability to learn the two-way active (shuttle box) avoidance response (see Escorihuela et al., 1995a, 1999). The RHA/RLA lines/strains are known to differ in many other respects, both at the behavioral and the neuroendocrine/neurochemical levels, with the RHA being less emotionally reactive to stressors and more active copers than the RLA rats (e.g. Driscoll et al., 1998). The effects of neonatal handling (see procedure in Escorihuela et al., 1995a) on different behavioral measures are shown in this table. (A) Timidity: Litters (4 weeks old) of each sex and similar number of animals (4–8) were tested for timidity by partially pulling their home cages (approximately 15 cm) out of the rack. Results (pooled for both sexes) are expressed as percentage of animals performing the indicated behaviors. (B) Dark–light hexagonal tunnel maze: Animals (5.5 weeks old) were introduced into the dark ring of the maze and tested for 6 min over 2 consecutive days. Mean ± S.E.M. are shown. Overall, RLA-I/Verh rats showed a higher latency to enter into the lit center, lower number of entries into this area and lower total activity as compared to RHA-I/Verh rats. Handling treatment decreased the latency and increased the entries into the center and the total activity in both strains. There were "strain" and "handling" effects in all variables across the 2 testing days [all F's$(1,39) \geq 8.4$ and $P \leq .006$]. (C) Dark–light box: Animals (7.5 weeks old) were placed in the dark compartment and observed for 5 min. Mean ± S.E.M. are shown. The number of entries into the lit compartment was reduced in RLA-I/Verh as compared to their RHA-I/Verh counterparts [two-way ANOVA "strain effect" $F(1,59) = 15.7$, $P < .001$]. Handling treatment increased both the number of entries into the lit compartment and the time spent in it [two-way ANOVA "handling effect" $F(1,59) = 6.9$, $P < .02$ and $F(1,59) = 9.1$, $P < .005$, respectively).

* $P < .02$ vs. RHA-I/Verh nonhandled group.
** $P < .02$ vs. RLA-I/Verh nonhandled group (χ^2).
*** $P < .05$ vs. RHA-I/Verh nonhandled group.
**** $P < .05$ vs. all groups.
$^{\#}$ $P < .05$ vs. RHA-I/Verh handled group (Duncan's tests).
† $P < .05$ vs. RLA-I/Verh nonhandled group.
‡ $P < .05$ vs. all groups.

and Ray, 1972; for a review, see Renner and Rosenzweig, 1987). An at least partial explanation for those inconclusive results could be that EE-treated animals show a different (i.e. a more complex and diverse) organization of exploratory behavior, as reported by Renner and Rosenzweig (Renner, 1987; Renner and Rosenzweig, 1986, 1987; Widman and Rosellini, 1990) in more detailed qualitative studies. Two other factors to be taken into account to explain those inconsistencies are the use of a variety of testing situations that may not be reflecting "pure" exploratory behavior (e.g. some open-field-like tests with differential degrees of aversiveness) (e.g. Fernández-Teruel et al., 1992a; Zimmermann et al., 2001) and the fact that, very often, EE studies compare animals reared in groups in an enriched environment with animals reared singly in a barren environment, thus socially isolated (e.g. Mohammed et al., 1993; Renner and Rosenzweig, 1987).

Although the consequences of EE on emotionality/anxiety have been rarely studied to date, indications are that, if anything, EE reduces fearfulness, as suggested by reductions of defecation in open-field-like tests (Fernández-Teruel et al., 1992a; Freeman and Ray, 1972; Manosevitz and Joel, 1973; Larsson et al., this issue) and by increases in the number of entries into (although not in the time spent in) the open arms of the elevated plus-maze (Escorihuela et al., 1994b; Fernández-Teruel et al., 1997). It is interesting to note, however, that in one study in which EE rats had also received EH, such a combined stimulation led to clear

Table 2
Effect of neonatal handling on stress-induced plasma hormone levels 20 min after a 5-min exposure to the open-field test

	RHA/Verh		RLA/Verh	
	Nonhandled ($n=16$)	Handled ($n=14$)	Nonhandled ($n=14$)	Handled ($n=16$)
Corticosterone (ng/ml)	292.3 ± 16.1	257.7 ± 8.5	331.1 ± 15.2[a]	317.0 ± 11.3[a,*]
ACTH (pg/ml)	1201.7 ± 77.4	1200.9 ± 83.1	1543.6 ± 91.7**	1298.0 ± 79.1

Animals were 22 months old. Mean ± S.E.M. are shown. The subjects were outbred RHA/Verh and RLA/Verh rats (see legend of Table 1 for details).
[a] Line effect, RHA/Verh vs. RLA/Verh [$F(1,59)=5.6$, $P<.002$].
* $P<.05$ vs. the RHA-I/Verh handled group.
** $P<.05$ vs. all the groups (Duncan's tests after significant ANOVA).

decreases of anxious behavior (i.e. higher percentages of entries and of time spent in the open arms) in the elevated plus-maze (Santucci et al., 1994). In tasks involving classical fear conditioning, there is some evidence of a lesser expression of fear-related responses in EE-treated rats (e.g. Nikolaev et al., in press; Larsson et al., this issue), whereas long-lasting improvements of two-way active avoidance acquisition have also been found in EE rats (Escorihuela et al., 1994a; Ray and Hochhauser, 1969). These results, although much less convincing than those that were mentioned for EH treatment, tend to indicate that EE may induce some "anxiolytic" effects (at least in some instances; see also below), which may be more pronounced when the tasks or situations used are highly challenging for the organism (e.g. the initial acquisition of two-way active avoidance).

Regarding the influences of EE treatment on endocrine responses to stressors, it must be acknowledged that, although EE animals appear to be less "behaviorally" stressed than control animals (see above), those results have not received additional support from hormonal findings, as HPA axis hormonal responses have not been shown to be differentiated in EE and nonenriched subjects (Devenport et al., 1992; Pham et al., 1999; Van de Weerd et al., 1997; see Renner and Rosenzweig, 1987; Larsson et al., this issue). Given that, when exposed to threatening situations/tasks, EE-exposed subjects usually show a superior ability to adapt or to cope when the situation is highly conflicting/stressful and/or must be solved by using complex strategies (e.g. in the initial acquisition of the two-way avoidance task), it would be tempting to speculate that in such tasks their hormonal responses could play a role and/or be different from those of nonenriched animals (although, as said above, experimental evidence supporting that hypothesis is still lacking). Besides the aforementioned EE effects on two-way avoidance acquisition (e.g. Escorihuela et al., 1994a), which could be seen as congruent with the above argument, another interesting insight along the same lines comes from the work of Klein et al. (1994). Those authors found that, compared with control rats, EE-treated rats exposed to the presence of a cat (i.e. predator stress) showed a clear decrease in a series of defensive responses (e.g. unconditioned and conditioned freezing, "proximity to/avoidance of" the predator compartment, latency to enter into and time spent in the dark compartment were the cat could be seen through a screen, etc.), thus indicating that the EE rats experienced less stress and/or fear and/or that they coped with it in a more active manner (e.g. showing less freezing, more approaches to the predator screen, etc.) than nonenriched (group-housed) rats (Klein et al., 1994). Although it cannot be completely ruled out that changes in learning or information processing abilities (due to EE) have influence on the initial acquisition of two-way avoidance (Escorihuela et al., 1994a) or in the behavior of EE rats in the predator stress experiment (Klein et al., 1994), most of the research emphasizes the role of fear/anxiety in these two experimental situations. The use of complex (or especially aversive) procedures like two-way avoidance or predator exposure could therefore be interesting approaches to evaluate the influence of EE on endocrine responses.

[Note: In line with what has been proposed above, in a study reported while the present review was in progress, Roy et al. (2001) have shown for the first time that enriched (BALB/c) mice presented a lower corticosterone response to a predatory cat odor (cat feces) than the respective nonenriched mice.]

2.3. Conclusion

There appear to be some similarities between the behavioral effects of EH and EE treatments, which, considering general behavioral (or psychological) constructs rather than the specific behavioral measures used, we could attempt to summarize as follows: (1) Both treatments tend to increase activity and/or specific exploratory behavior in tests involving novelty (e.g. open-field, hole-board, etc.), although the quality, diversity or organization of exploratory behavior could differ between EE and EH, probably being more complex in the former case. (2) Emotionality/fearfulness (as measured, for instance, by novelty-induced defecation, dark–light tests and elevated plus-maze) is more clearly reduced by EH, although some results in the same direction have been reported for EE. (3) Of particular interest is the fact that in an anxiety-mediated aversive learning task, such as the initial acquisition of two-way active avoidance, both treat-

ments show a similar pattern of improving effects (without increasing general activity), leading to the conclusion that anxiety (or conditioned fear) is reduced in both cases.

Concerning basal and stress-induced hormonal responses, it is clear that EH has a long-lasting, facilitating and protective effect on HPA axis function, whereas the studies carried out thus far do not show consistent effects of EE on HPA axis responses (being the only exception to date is the recent study by Roy et al., 2001) or a role for these hormones in the behavioral and neurobiological actions of EE.

3. Effects of EH and EE on learning and memory processes

EH has been shown to improve either acquisition or memory function in various learning tasks (but see also Daly, 1973), such as passive avoidance (e.g. Gschanes et al., 1998; Wong, 1972), T-mazes (Wong and Jamieson, 1968), spatial orientation in the Morris water maze (Aguilar et al., 2002b; Meaney et al., 1988; Pham et al., 1997; Zaharia et al., 1996) and two-way active avoidance (e.g. Escorihuela et al., 1991, 1992, 1994a; Levine, 1956; Levine and Wetzel, 1963; Núñez et al., 1995). Absence of effects of EH or otherwise inconsistent results have been most commonly reported when the tasks used are relatively simple or involve less motivational/emotional factors (see, for instance, Daly 1973; Denenberg and Zarrow 1971), although others have reported that EH and even postweaning handling is able to improve latent inhibition and prepulse inhibition in rats (Krebs-Thompson et al., 2001; Peters et al., 1991).

EE also improves acquisition and/or retention in several learning tasks, ranging from spatial and/or problem-solving tasks (e.g. Bennett et al., 1970; Cooper and Zubek, 1958; Denenberg et al., 1968; Denenberg and Morton, 1962b; Forgays and Read, 1962; Freeman and Ray, 1972; Juraska et al., 1980; Kempermann et al. 1997; Liljequist et al., 1993; Mohammed et al., 1990; Paylor et al., 1992; Ray and Hochhauser, 1969; Smith, 1972; Venable et al., 1988; Woods, 1959; reviewed by Escorihuela et al., 1994b; Renner and Rosenzweig, 1987) to the acquisition and long-term retention of two-way active avoidance (Escorihuela et al., 1994a). Likewise, EE has been shown to also improve retention in nonspatial tasks and object recognition tests (Escorihuela et al., 1995c; Rampon et al., 2000). Nevertheless, there are also reports of no effects of EE when the tasks used are relatively simple, as for instance habituation (but see also Larsson et al., this issue), visual discrimination, passive avoidance or some tasks of visual discrimination (e.g. Bernstein, 1973; Davenport, 1976; Freeman and Ray, 1972; Krech et al., 1962; Lore, 1969; Sjoden, 1976; see review by Renner and Roseznweig, 1987).

It appears, therefore, that both EH and EE treatments improve learning most consistently when the tasks used have a relatively high level of aversiveness and/or complexity, as do some spatial tasks (the most commonly used being the Morris water maze) and the two-way active avoidance task. In addition, the influences of both treatments on learning ability appear to be lifelong (e.g. Meaney et al., 1988, 1991; Fernández-Teruel et al., 1997; Renner and Rosenzweig, 1987).

4. Effects of EH and EE on novelty/sensation seeking

In laboratory rodents (and this is especially clear in rats), responses to novelty are ambivalent, as the desire to explore novel/unknown environments is in competition with the tendency to avoid them on the basis of fearfulness or neophobia. Therefore, outcomes of activity and exploratory behavior in laboratory rodents exposed to different degrees of novelty have to be interpreted with caution, as the meaning of the results can easily change as a function of the degree of novelty involved in the specific testing situation and a function of the environmental and/or biological background/history of the animals (e.g. Bardo et al., 1996; Dellu et al., 1996; Gentsch et al., 1991; Zimmermann et al., 2001).

The most common and current view of what is emotionality and exploratory activity in rodents is that these two constructs represent independent dimensions rather than extremes of the same variable, although the outcomes of factor analytic studies also indicate that both dimensions are internally complex (e.g. Aguilar et al., 2002a; Fernandes et al., 1999). Moreover, experimental approaches to that issue also tend to support the idea of a separation or independency between those two constructs (e.g. Abel, 1995; Dellu et al., 1996; Fernández-Teruel et al., 1992a; Gentsch et al., 1991; Zimmermann et al., 2001). Thus, experimental studies appear to agree in that directed exploration or ‘‘novelty preference/novelty seeking’’ is mainly reflected by conditions in which an animal can make an unforced choice between the novel/unfamiliar elements (e.g. objects or spaces) of the situation and those which are more familiar or present fewer aspects of novelty (e.g. Abel, 1995; Dellu et al., 1996; Escorihuela et al., 1999; Fernández-Teruel et al., 1992a; File, 1978; File and Wardill, 1975; Zimmermann et al., 2001). Hence, studies on the influences of EH and EE treatments on different types of exploratory behaviors should take into consideration (1) which variables are considered to reflect exploration rather than pure, unspecific locomotor activity and (2) when and under which experimental conditions exploratory behavior can be considered to be congruent with novelty preference/novelty seeking rather than emotionality.

It is therefore understandable that frequent inconsistencies are to be found in the literature on the effects of EH and EE upon different measures of activity/exploration in a variety of novelty tasks (see references in Section 2 above and see also Escorihuela et al., 1995a; Fernández-Teruel et al., 1992a, 1997; Larsson et al., this issue; Renner and Rosenzweig, 1987; Zimmermann et al., 2001). How-

ever, if we focus upon studies which measure what can actually be considered to be novelty preference or novelty seeking, then the results would indicate that EH tends to increase novelty preference in some cases (e.g. De Nelsky and Denenberg, 1967; Fernández-Teruel et al., 1992a; Ferré et al., 1995; File, 1978; Núñez et al., 1995; Steimer et al., 1998; see also "exploration of a novel object" in Table 1A and the difference in "latency to enter the illuminated center" between days 1 and 2 in Table 1B). Interestingly, however, in several experiments performed at our laboratory (Escorihuela et al., 1994b), we introduced EH-exposed rats into large environmentally enriched cages containing a variety of novel stimulus objects in only one half-side of the cage that was separated by an opaque panel from the opposite side (not containing objects), where the animals were confined for 5–10 min. When that panel was pulled out a few (approximately 15) centimeters to allow the animals to enter in the enriched side of the cages, it was observed that EH rats entered that side and started to explore the objects much faster than unhandled rats (Escorihuela et al., 1994b).

On the other hand, novelty preference, and the complexity of exploratory behavior in which animals engage, appears to be clearly enhanced in EE rats (e.g. Escorihuela et al., 1995c; Fernández-Teruel et al., 1992a; Larsson et al., this issue; Renner 1987; Renner and Rosenzweig, 1986, 1987; Widman and Rosellini, 1990), although faster habituation to novelty (i.e. faster within-session decrease of activity and exploration of novel objects) has also been found as a consequence of EE (Zimmermann et al., 2001), thus suggesting that improved spatial abilities (due to EE) could explain at least some of the findings of EE effects on behavior under novelty conditions (Zimmermann et al., 2001).

5. Neurobiological consequences of EH and EE

Both treatments have been found to have profound and long-lasting neural and physiological consequences. EH has been shown to induce (1) increases in hippocampal but not cortical 5-HT and 5-HIAA in rats (e.g. Anisman et al., 1998 for a review; Núñez, 1997; see also Table 3), being that effect specific for "consistent EH" but not for "inconsistent/stressful mild stimulation" administered during the same period (see Escorihuela et al., 1991, 1992, 1994a; Núñez, 1997) (Table 3); (2) increases in stress-induced dopamine content in the nucleus accumbens of mice (see Anisman et al., 1998 for review); (3) probable changes in cyclic AMP (Anisman et al., 1998; Escorihuela et al., 1995c), which could be related to the effects observed on 5-HT (see Anisman et al., 1998); (4) increases in hippocampal ornithine decarboxylase (after a dexamethasone challenge) (Gilad et al., 2000); (5) increases in the magnitude of hippocampal long-term potentiation in young rats (Wilson et al., 1986); (6) decreases of HPA axis responses to stress (e.g. Meaney et al., 1988; Núñez et al., 1996) linked to enhanced hippocampal type II glucocorticoid (GC) receptors (e.g. Meaney et al., 1988); (7) increases of hippocampal nerve growth factor (NGF) mRNA (Mohammed et al., 1993; Pham et al., 1997); (8) increases in brain benzodiazepine (BZ) and GABA-A receptors (Bodnoff et al., 1987; Bolden et al., 1990; Escorihuela et al., 1992) and up-regulation of peripheral BZ receptors in adrenals and kidney (Weizman et al., 1999); (9) enhancement of NADPH-diaphorase-positive neurons (a potential marker of nitric oxide-producing neurons) (Vaid et al., 1997); (10) apparent attenuation of NMDA-induced convulsions and death in psychogenetically selected rats (RLA rats) (see Table 4); (11) increases in cortical dendritic spines (reviewed by Pham et al., 1999) and (12) no changes on cortical phospholipase-C β1 levels (associated to muscarinic receptors) (Fernández-Teruel et al., 2000; Sallés et al., 1993).

Table 3
Effect of neonatal handling (consistent and inconsistent) (see text and Escorihuela et al., 1991, 1992 for procedures) on the 5-HIAA and 5-HT levels in cortex and hippocampus of adult Sprague–Dawley rats

	Sprague–Dawley		
	Nonhandled ($n=12$)	Handled ($n=6$)	Inconsistently handled ($n=6$)
Cortex			
5-HIAA (ng/g)	259.5 ± 10.1	291.5 ± 18.4	275.7 ± 18.6
5-HT (ng/g)	400.0 ± 17.1	414.0 ± 27.8	393.0 ± 19.4
Hippocampus			
5-HIAA (ng/g)	270.8 ± 12.3	319.9 ± 21.8 *	273.1 ± 30.9
5-HT (ng/g)	262.6 ± 10.3	321.4 ± 8.9 *	294.7 ± 20.7

Mean ± S.E.M. are shown.
* $P<.05$ vs. the respective nonhandled group (Duncan's tests) (adapted from Núñez, 1997).

For its part, EE has been shown to induce (1) higher levels of acetylcholinesterase activity in subcortical and cortical brain regions (e.g. Pögun et al., 1992; Por et al., 1982; Renner and Rosenzweig, 1987); (2) higher hippocampal expression of the gene for the 5-HT1A receptor (Rasmuson et al., 1998); (3) decreased β-adrenoceptor-linked cyclic AMP accumulation in the hippocampus (an effect that parallels that of some pharmacological treatments, which improve age-related cognitive deficits) (see Escorihuela et al., 1995c); (4) higher hippocampal expression of NGF and NGF-induced immediate early gene factor (NGFIA) (even after a brief exposure to EE in weanling rats) (see Mohammed et al., 1993 and references therein); (5) higher hippocampal expression of GC type II receptor mRNA (Mohammed et al., 1993); (6) greater excitatory postsynaptic potential (EPSP) slopes in the dentate gyrus (Foster et al., 2000; Green and Greenough, 1986), enhanced hippocampal field potentials (Sharp et al., 1985, 1987) and long-term potentiation (Hargreaves et al., 1992); (7) increased hippocampal protein kinase C (PKC) activity after only a 12-day exposure to EE in very young rats (between postnatal days 15 and 27) (Paylor et al., 1992); (8) increased

Table 4
Effect of EH on NMDA-induced convulsant seizures and death

	Convulsant seizure			Mortality		
	Nonhandled (%)	Handled (%)	Protection (%)	Nonhandled (%)	Handled (%)	Protection (%)
RHA-I/Verh						
Saline	0	0	–	0	0	–
NMDA (30 mg/kg)	0	0	–	0	0	–
NMDA (60 mg/kg)	42.9	0	100	14.3	0	100
NMDA (120 mg/kg)	12.5	14.3	0	12.5	14.3	0
RLA-I/Verh						
Saline	0	0	–	0	0	–
NMDA (15 mg/kg)	50	14.3	71.4	0	0	–
NMDA (30 mg/kg)	50	57.1	0	0	0	–
NMDA (60 mg/kg)	62.5	42.9	31.4	25	28.6	0
NMDA (120 mg/kg)	71.4*	57.1*	20	71.4*	28.6*	60

Two-month-old female rats were injected intraperitoneally with different doses of the glutamatergic agonist and observed for 1 h. The percentages of animals exhibiting seizure or mortality and protective effect of EH are shown. $n=6$ (saline controls) or $n=7-8$ (NMDA injected).

* $P<.03$ between nonhandled and handled groups (one-tailed Mann–Whitney U-test on the latency of convulsions).

brain RNA content (e.g. Renner and Rosenzweig, 1987; Rosenzweig and Bennett, 1996) and changes in the expression level of a large number of genes (many of which can be linked to neuronal structure and synaptic function and plasticity) (Rampon et al., 2000); (9) morphological alterations in hippocampus and cortex that include increases in thickness, glial proliferation, perikarya and nuclear dimensions, dendritic branching and spine counts and higher synaptic density (e.g. for reviews, see Diamond, 1988; Renner and Rosenzweig, 1987; Rosenzweig and Bennett, 1996) and (10) hippocampal neurogenesis in adult animals (Kempermann et al., 1997; Van Praag et al., 2000).

At first glance, this overview of EH and EE neurobiological effects would appear to suggest very few connections between the two treatments, although this could be partially due to the focus of research interests (i.e. the main variables/parameters studied for each treatment), having been divergent from the beginning (already in the 1950s and 1960s) of these two paths of research. Nevertheless, some interesting similarities arise as, for instance, both EH and EE increase hippocampal type II GC receptors and NGF (e.g. Meaney et al., 1988; Mohammed et al., 1993) and both treatments appear to facilitate hippocampal synaptic transmission and plasticity (including long-term potentiation) (e.g. Hargreaves et al., 1992; Sharp et al., 1985; Wilson et al., 1986). These possible commonalities are discussed in Section 6.

6. EH and EE prevent age-related impairments: are there some common mechanisms for their behavioral and neurobiological effects?

6.1. Effects on age-related impairments

In rats and in parallel to a long-lasting attenuation of emotional responses to stressors (although in the case of EE caution is still required, because as mentioned in previous sections the evidence is still far from conclusive), both EH and EE prevent the age-related impairments of spatial learning/memory (e.g. Escorihuela et al., 1995c; Fernández-Teruel et al., 1997; Meaney et al., 1988, 1991; Steimer et al., 1998) and increase the expression of hippocampal GC (type II) receptors. Research with EH indicates that the effect on GC receptors is a likely consequence of a reduction of HPA axis hormonal responses (Meaney et al., 1988, 1991; Mohammed et al., 1993; see also Larsson et al., this issue) (Table 2), whereas the issue is still open concerning EE, because there is no evidence of altered HPA axis activity in EE rats although it has actually been reported very recently in mice (Roy et al., 2001).

Related to that and concerning the consequences of these forms of stimulation on the aging brain, it has been shown that EH prevents age-related neurodegeneration in the hippocampus of nonselected rats and of psychogenetically selected lines of rats (Meaney et al., 1988; Fernández-Teruel et al., 1997). Furthermore, work from our laboratory suggests that such a neuroprotective effect is also produced by EE treatment and when both EH and EE are administered together (Fernández-Teruel et al., 1997), which in turn appears to induce glial proliferation (González et al., 1994; Renner and Rosenzweig, 1987). Whether these neuroprotective effects of both EH and EE can be explained by a reduced exposure of the hippocampus to GCs (Meaney et al., 1988, 1991) remains to be elucidated, although the results of Steimer et al. (1998) and those of Table 2 would tend to be congruent with that contention, at least in regard to EH.

Although no differences in either basal or stress-induced corticosterone levels have been thus far reported in EE-treated rats (reviewed by Larsson et al., this issue), it appears to be a plausible hypothesis that EE rats could have a more adaptative HPA axis system, as they show (in a way similar to EH-exposed rats) elevated expression of GC

receptors in the hippocampus, which probably provide them with a more efficient negative feedback mechanism in that system (behavioral experiments lend support to this idea) (e.g. Larsson et al., this issue). This, in turn, should make EE animals more resistant to the effects of stressors and less vulnerable to the deleterious neurotoxic processes that prolonged exposure to high levels of GCs can potentiate at the hippocampal level (Meaney et al., 1988, 1991; Mohammed et al., 1993; Sapolsky, 1992). It should be acknowledged, however, that there are only two indirect findings, which could lead some credibility to such a hypothesis in regard to EE, the first being the aforementioned changes in hippocampal glucocorticoid receptor expression in EE rats (Mohammed et al., 1993) and the second being the recent finding of a lower corticosterone response in enriched BALB/c mice exposed to a (predatory) cat odor (Roy et al., 2001). Thus, that hypothetical reasoning remains essentially speculative and pending of experimental demonstration.

6.2. Insights into shared effects

In spite of the intrinsic potency of factorial (2×2) experimental designs for the study of interactions among treatments and thus for the elucidation of possible shared mechanisms, very few studies have been performed in which EH and EE are simultaneously compared and/or administered in combination (i.e. in the form of a factorial design). Of these, some reports indicate that positive EH and EE effects on both spatial and aversively motivated learning tasks appear to be additive or, in any case, not interactive (Escorihuela et al., 1994a, 1995b,c; Fernández-Teruel et al., 1997; Pham et al., 1999), although some EH $\times$ EE interactions have been found in reversal spatial tasks in aged animals (Escorihuela et al., 1995b). Additivity or absence of interactions is also the common result when considering the consequences of EH and/or EE (alone or combined) on emotional or fear-related behavior (Denenberg et al., 1978; Fernández-Teruel et al., 1992a,b; Garbanatti et al., 1983; Jones et al., 1991) and on novelty-seeking/preference (Fernández-Teruel et al., 1992a). However, a study by Denenberg et al. (1978) deserves mention, in which clear interactions between both treatments appeared with respect to open-field activity in right brain-lesioned rats. Likewise, Pham et al. (1999) reported interactive EH $\times$ EE effects on hippocampal NGF levels (which are otherwise increased by both treatments) (see previous sections) (Pham et al., 1997, 1999).

Given the scant evidence of EH $\times$ EE interactions reported thus far, which might suggest that both treatments act upon different mechanisms, other possible factors should be first taken into consideration. Interaction effects (by applying ANOVA) are much more difficult to realize, primarily because of the mathematical characteristics of ANOVA that clearly favor the appearance of main factor effects (Wahlsten, 1990). This is especially true when some of the main factors are very potent (for instance, by having many levels) and predictable in regard to the direction of effects, for example, when (as is the case in many learning or "repeated testing" experiments) there is a "trial" and/or "testing day" within-subject factor or when there are several (more than two) between-subject factors that can have even opposite effects on a given variable (for examples of this, see Escorihuela et al., 1994a, 1995b,c; Pham et al., 1999; Wahlsten, 1990). Hence, it is quite possible that designs studying EH $\times$ EE effects do not show interactive effects because these remain "hidden" (because of the aforementioned reasons) even if they may actually exist.

Thus, with the available information obtained from factorial experimental designs (combining EH and EE treatments), it is difficult to make a general statement about the outcomes of these treatments. Therefore, returning once again to studies that have separately examined EH or EE effects, the data tend to support the idea that both EH and EE enhance exploration under novelty conditions. Novelty by itself (as that involved in the enriched environments) and neonatal handling are mildly stressful conditions that produce HPA axis responses (e.g. increases in circulating corticosterone levels), and moderate increases in corticosterone levels are known to improve learning/memory processes (e.g. Denenberg, 1975; Larsson et al., this issue; Sandi, 1998; Sharp et al., 1985). This leads to the logical hypothesis that EH and EE should be able to produce some kind of facilitation/potentiation or plastic effects on neural mechanisms, subserving a role on the type of cognitive processes that both treatments improve (e.g. several types of learning). In fact, although the evidence is still scarce, there are indications that EH and EE facilitate synaptic processes at the hippocampal level, including LTP, even in young rats (Green and Greenough, 1986; Hargreaves et al., 1992; Sharp et al., 1985, 1987; Paylor et al., 1992; Wilson et al., 1986). Interestingly, such a facilitation of hippocampal synaptic responses seem to be paralleled by EH- and EE-induced increases in the expression of NGF in the same area and of glucocorticoid (type II) receptors (Meaney et al., 1988, 1991; Mohammed et al., 1993; Pham et al., 1997, 1999). These are striking parallelisms, as memory impairments in aged rats have been associated with impaired hippocampal synaptic plasticity, reduced NGF levels, decreased GC receptor levels and hippocampal neurodegeneration (reviewed by Mohammed et al., 1993). It is conceivable, therefore, that NGF elevation produced by EH and EE and/or the apparent optimization of GC-related neural processes induced by the treatments (see Meaney et al., 1988; Mohammed et al., 1993; Sapolsky, 1992) could help to maintain hippocampal synaptic plasticity, learning/memory function, better ability to adapt to (or to cope with) stress or conflict and protection from age-related emotional or cognitive deficits and neurodegeneration (e.g. Mohammed et al., 1993; Pham et al., 1999; Sapolsky, 1992).

The issue of whether some of those effects can be considered as actual mechanisms still needs much further

research. The precise ontogenetic stage in which some of those treatment effects are detected is of great relevance. In this sense, it is worth noting, for instance, that EH-induced corticosterone elevations were observed in 2-day-old rats (e.g. Denenberg, 1975), whereas the appearance of EH effects (on HPA axis responses and hippocampal GC receptors) have been shown to already partly depend upon the integrity of brain 5-HT systems in the neonatal period (reviewed by Anisman et al., 1998). Also interestingly, some hippocampal plastic synaptic effects of EH have been observed in young rats (around weanling age) (Paylor et al., 1992; Wilson et al., 1986). As these phenomena could be related to glutamatergic transmission (e.g. NMDA receptors), the study of the role of such a neurotransmitter system on EH and EE effects at very early ages seems warranted. In this sense, there are indications that glutamate can play a role in EH effects, specifically because of the attenuation of the "GC–glutamate–calcium" neurotoxic cascade (Sapolsky, 1992) and there is also suggestive evidence for a EH-induced protection against convulsions and death produced by NMDA in young rats from the RHA and RLA strains (see Table 4). Enrichment effects on spatial learning have also been shown to be mediated by glutamatergic NMDA receptors (Liljequist et al., 1993).

6.3. A consideration of unshared neural and behavioral effects

Although, as noted above, some similar behavioral and neural consequences have been found after EH and EE, that picture would be overly simplistic if we do not take into account that many differential effects of both treatments have been reported along the past 50 years. If one should quickly summarize the main findings reported in EH and EE studies, it becomes clear that emotionality/anxiety/stress and GC-associated processes would appear among the most prominent aspects affected by EH (e.g. Denenberg, 1975; Fernández-Teruel et al., 1997; Levine, 1957; Levine et al., 1957, 1967; Meaney et al., 1988, 1991), whereas learning, physical activity and/or interaction with the enriched environment, information processing and plasticity-related neural effects (i.e. neurogenesis, synapse formation, dendritic branching, etc.) would seem as the main processes influenced by EE (e.g. Bennett et al., 1970; Mohammed et al., 1993; Renner and Rosenzweig, 1987; Rosenzweig, 1979; Van Praag et al., 2000). Thus, because most behavioral (including learning) tests involve emotional/stress responses to a some extent but also depend upon information processing, it is quite possible that both manipulations (EH and EE) could lead to similar behavioral effects even by affecting different underlying neural mechanisms (e.g. enhanced learning of some tasks may be due to relatively low stress levels due to EH or enhanced information processing due to EE). Similarly, neurobiological phenomena such as neurodegeneration and age-related cognitive decline or their prevention could be the outcome of fundamentally different processes, either related to changes in chronic stress levels (and stress-induced neural damage) in the case of EH or related to changes in the levels of sensory and cognitive stimulation (known to affect neural plasticity) in the case of EE.

Alternative hypotheses, however, such as the fact that EH could also affect (already in very early stages of development) information processing, should not be completely ruled out, because in most cases that treatment involves not only handling but also repeated exposure of the pups to a novel cage lined with paper towel (so allowing animals to explore new environments) for some minutes daily. In addition, hippocampal long-term potentiation has been shown to be facilitated in young EH rats (Wilson et al., 1993). Nevertheless, from a behavioral standpoint, that hypothesis should be tested in very young animals while receiving the EH treatment and using tests devoid (as much as possible) of emotional components.

On the other hand, as mentioned in previous sections, the finding that under some testing conditions EE mice showed reduced corticosterone levels (Roy et al., 2001) leaves the possibility open to more exhaustively explore whether that finding extends to other experimental situations and species (e.g. laboratory rats) and whether it is (or it is not) related to the changes in hippocampal GC receptors reported in EE rats (Mohammed et al., 1993).

In conclusion, it is clear that the available literature indicates much less commonalities than differences between the neurobehavioral effects of EH and EE, as it could be expected by the apparently extreme differences in the variables that are manipulated when administering each treatment. It is striking, nevertheless, that some similarities can be found between them, and it has been the aim of this work to revise and summarize them. They could be, as said above, the outcomes of essentially differential processes affected by EH and EE but, in any case, are worth investigating.

Studies parametrically combining both EH and EE (in factorial designs), administered at very early ages and simultaneously (at the same age), concurrently combined with candidate neurobiological/physiological measures that could mediate the appearance of EH/EE effects, are necessary if we aim to better elucidate which are possible (and possibly common) mechanisms and which are merely part of the constellation of effects of the treatments.

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