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Age, experience, and neurobehavioral domain: dissociable effects of environmental enrichment and social isolation on brain and behavior in adult and aged rats

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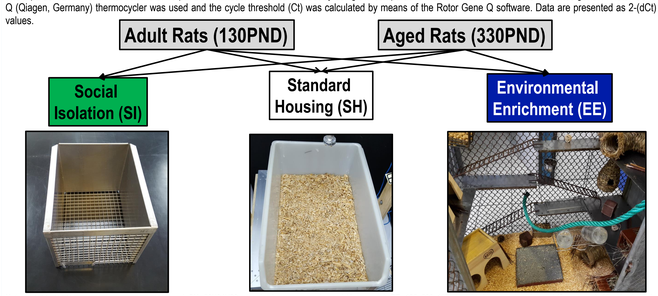
Introduction

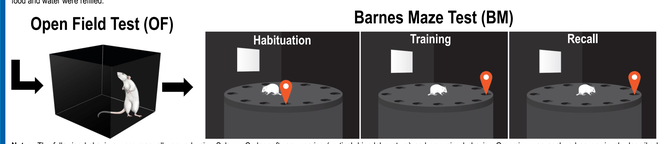
Environmental enrichment (EE) is a form of physical and social stimulation in rodents to model the effects of optimal developmental conditions, whereas social isolation (SI) constitutes a form sensorial and social deprivation used to study the effects of environmental impoverishment. When implemented during early life, these housing conditions deeply impact the animals phenotype: EE induces a pattern of more efficient responses to stress and increased cognitive capabilities, whereas SI induces the opposite effects. Because sensitive windows for neurobehavioral plasticity become narrower as organisms grow up, it is still unclear how much positive or negative experiences throughout adulthood are still able to improve or compromise behavioral, emotional, and cognitive domains, and neural plasticity. To address this question, we studied the behavioral effect of EE and SI on adult and middle-aged individuals in the Open-Field Test (OF) and the Barnes Maze Test (BM). OF consists of an open, illuminated, and inescapable arena that naturally increases the emission of exploratory and risk-assessment behaviors (E&RA) in rodents. When animals learn that there is no actual threat, the emission of E&RA decrease while other non-defensive behaviors —such as self-grooming— start to increase. Thus, we used the OF to assess both defensive and non-defensive strategies employed to cope with the mild aversiveness of the context. BM consists of an elevated, circular platform with holes radially distributed closed to the edge. One of the holes has a basket underneath allowing the animals to escape form the platform. Rats find the hole by using spatial cues distributed around the maze. Learning is noted when latencies to escape reduce over trials, which are indicator of spatial memory performance. After behavioral testing, we measured in the nucleus accumbens (NAcc) and the ventral (vHPC) and dorsal hippocampus (dHPC) the expression of genes associated with neural plasticity, such as the brain-derived neurotrophic factor (BDNF), the cyclic adenosine monophosphate response element binding protein (CREB), the p250GAP, the cofilin, the glutamate AMPA receptor subunits GluA1 and GluA2, and the glutamate NMDA receptor NR2B. We also studied the expression of the DNA methyltranspherase 3A (DNMT3A) as a global indicator of epigenetic changes.

Materials and Methods

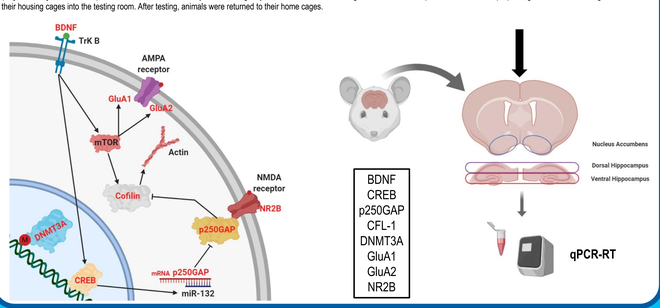
Subjects: We used a total of 48 male Wistar rats (LEBi, UCR) divided in adults (n=24, 130 PND) and aged individuals (n=24, 330 PND). Animals were behaviorally screened in a spontaneous activity test (cage-test) and according to the levels of exploratory activity they were assigned to the groups in a counterbalanced manner. **Groups:** Animals were housed for 50 days either on social isolation (SI), environmental enrichment (EE) or under standard housing conditions (SH). **General procedure:** After the housing period, animals were tested on two consecutive days in the OF (55 x 55 x 40cm illuminated at ~10 lumens) for 20 min. On the next day, rats were tested in the Barnes Maze Test (BM, 150x150cm; illuminated at ~550 lumens). BM consisted of three phases: (1) *Habituation:* animals were taught how to escape from the bright platform trough one of the holes. Afterwards, they had one single trial. (2) *Training:* On the next day, the escape hole was relocated. Then, rats were tested in four, ~2-h apart trials during 4 min each. There, animals had to find the escape hole using the spatial clues placed around the maze. (2) *Recall:* On the next day, the procedure of day 2 was repeated. **mRNA analysis**: One week after behavioral testing, animals were beheaded and their brain removed for a rapid dissection of their unilateral ventral (**vHPC**) and dorsal hippocampus (**dHPC**) and its nucleus accumbens (**NAcc**). Tissue was homogenized during 20 s in 300 µL of TRIzol and then it was stored at -70°C for further processing. RNA was extracted according to manufacturer's specifications, and quantified by using a nanodrop (Thermo Scientific, USA). Once extracted, a reverse transcription was run with RevertAid First Strand cDNA Synthesis Kit (Fermentas, USA) using the oligo dT method. RNA quantification was carried out by RT-qPCR. Amplification was done with 5 µl 2x QuantiTect SYBR Green PCR Master Mix, 75-150 ηmol primer and 2 µL del cDNA, for a final volume of 10 µL. Determinations were carried out by using the comparative method, with HPRT1 as the reference gene. A Rotor Gene Q (Qiagen, Germany) thermocycler was used and the cycle threshold (Ct) was calculated by means of the Rotor Gene Q software. Data are presented as 2-(dCt) values.



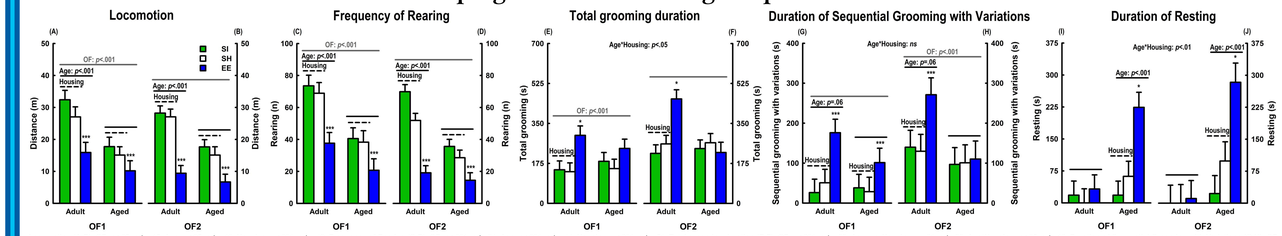
Notes: SI: 24x18x18cm; animals were singled housed. **SH:** 56X34X20cm; animals were group housed (4 per cage). **EE:** 135x568x110cm, animals were group-housed (8 per cage). All animals had free access to food and water, and were kept in a dark-light cycle of 12:12h with lights on at 6:00 h. Twice per week during bed changes objects and materials were replaced and rearranged and fresh food and water were refilled.



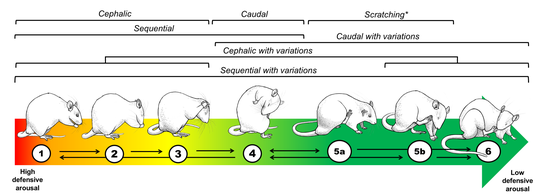
Notes: The following behaviors were manually scored using SolomonCoder software: rearing (vertical, bipedal posture) and grooming behavior. Grooming was analyzed as previously described (**Rojas-Carvajal et al., 2016**). Locomotion was automatically scored using AnyMaze® software. For testing, animals were transported in individual, opaque cages filled with bedding material from their housing cages into the testing room. After testing, animals were returned to their home cages.

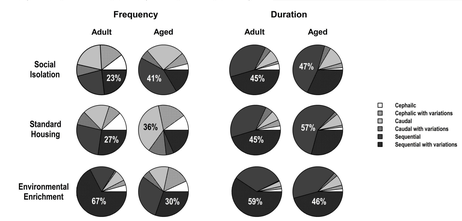


Results – EE reduced exploratory and risk-assessment behaviors irrespective of the age, while increased post-stress coping behaviors in an age-dependent manner.



Locomotion (A-B): OF: $p<.05$, η_p^2=. 15. **Age:** $p<.05$, η_p^2=. 40. **Housing:** $p<.001$, η_p^2=. 49. **Frequency of Rearing (C-D): OF:** $p<.001$, η_p^2=.34; **Age:** $p<.001$, η_p^2=. 44. **Housing:** $p<.001$, η_p^2=. 51. **Total grooming duration (E-F): OF:** $p<.001$, η_p^2=. 33; **Age:** *ns*. **Housing:** p=.01, η_p^2=.23. **Age*Housing:** $p<.05$, η_p^2=.16. **Duration of Sequential Grooming with Variations (G-H): OF:** $p<.001$, η_p^2=.31. **Age:** p=.06, η_p^2=. 09. **Housing:** p=.06, η_p^2=. 13; EE > SI AND SH ($p<.05$). **Duration of Resting (I-J): OF:** *ns*; **Age:** $p<.001$, η_p^2=. 35. **Housing:** $p<.001$, η_p^2=. 26. **Age*Housing**: $p<.01$, η_p^2=. 26. ***: $p<.05$; ***: $p<.001$.**



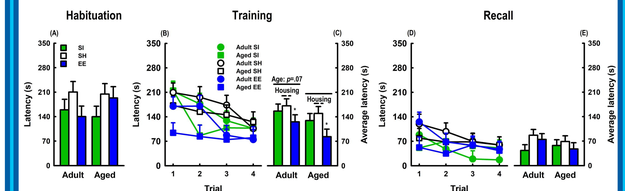


Frequency per subtype: Housing: *ns*. **Age** $p<.05$, η_p^2=. 007. **Subtype:** $p<.001$, η_p^2=.13. **Housing*Age:** $p<.05$, η_p^2=.015. **Housing*Subtype:** $p<.001$, η_p^2=.09. **Age*Subtype** $p<.001$, η_p^2=.05. **Housing*Age*Subtype:** $p<.001$, η_p^2=.06. **Duration per subtype: Housing:** $p<.01$, η_p^2=. 02. **Age**: *ns*. **Subtype:** $p<.001$, η_p^2=.41. **Housing*Age:** $p<.05$, η_p^2=.01. **Housing*Subtype:** $p<.001$, η_p^2=.08. **Age*Subtype:** $p<.001$, η_p^2=.04. **Housing*Age*Subtype:** *ns*.

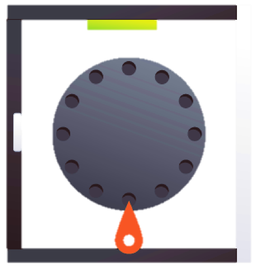
Main findings

- As expected, there was reduction of locomotion and rearing between OF1 and OF2 in all groups.
- Aged animals displayed less OF activity than adult animals.
- EE reduced locomotion and rearing in both age groups, whereas SI produced no alterations at all.
- Total grooming increased from OF1 to OF2 in all animals.
- EE increased total grooming but only in adult animals. Out of the different grooming subtypes, the sequential grooming with variations was the one that increased more substantially after EE.
- Aged animals did less grooming events per subtype as compared with adult animals, but spent about the same amount of time on them.
- In SI and SH animals, sequential grooming was the most frequent and time-consuming grooming subtype. All EE animals, but especially adults, displayed more and longer bouts of sequential grooming with variations.
- Overall, sequential grooming with or without variations was the most frequent and time-consuming grooming subtype.
- Interestingly, in aged rats EE caused a strong increase in the resting time.

Results – EE slightly improved spatial memory in both age groups, whereas SI produced no impairments.



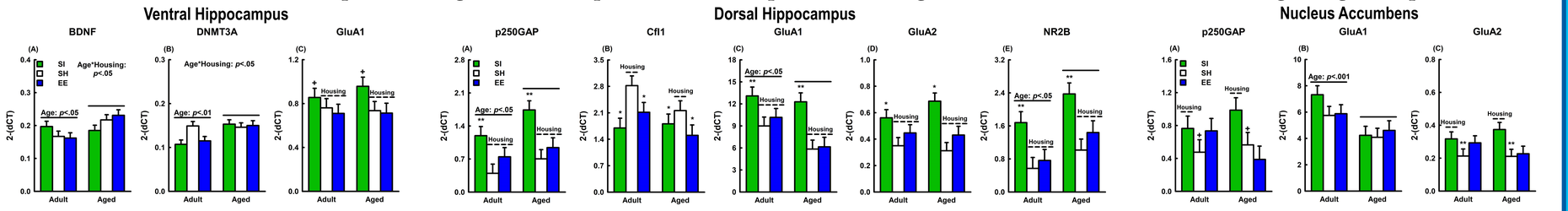
Habituation: Housing: *ns*. **Training: Trial:** $F(3,123)$=12.21, $p<.001$, η_p^2=.23; **Housing:** $F(2,41)$=3.91, $p<.05$, η_p^2=.16; **Age**: $F(1,41)$=3.91, p=.07, η_p^2=.08. **Recall: Trial:** $F(1.92,78.90)$=3.73, $p<.05$, η_p^2=.08; **Housing:** *ns*; **Age**: *ns*.



Main findings:

- We found no differences between housing conditions during the habituation phase.
- During the training and recall phases, a progressive reduction in the escape latency across trials was observed.
- During the training phase, EE animals of both ages showed the shortest escape latencies as compared with SH animals.
- We found no Age*Housing interaction, however, EE aged rats showed the shortest escape latencies from the first trial onwards.
- During the recall phase, no significant differences appeared. In general, younger animals did not outperform the older ones.
- SI caused no impairments on spatial learning at any time point in testing.

Results – SI increased the expression of glutamate receptor subunits irrespective of the age, whereas EE caused almost no changes in gene expression.



Ventral Hippocampus: BDNF: Age: $p<.01$, η_p^2=. 16. **Housing:** *ns*; **Age*Housing**: $p<.05$, η_p^2=. 15. **DNMT3A: Age:** $p<.01$, η_p^2=.20. **Housing:** *ns*; **Age*Housing**: $p<.05$, η_p^2=. 14. **GluA1: Age:** *ns*. **Housing:** p=.06, η_p^2=. 13; **Age*Housing**: ns. **Dorsal Hippocampus: p250GAP: Age:** $p<.05$, η_p^2=. 10. **Housing:** $p<.05$, η_p^2=. 10. **Age*Housing**: *ns*. **Cfl1: Age:** p=.08, η_p^2=. 07. **Housing:** $p<.05$, η_p^2=. 19; **Age*Housing**: *ns*. **GluA1: Age:** $p<.05$, η_p^2=.15. **Housing:** $F(2,41)$=10.96, $p<.001$, η_p^2=.35; **Age*Housing**: ns. **GluA2: Age:** *ns*. **Housing:** $p<.001$, η_p^2=.36; **Age*Housing**: ns. **NR2B: Age:** $p<.01$, η_p^2=.16. **Housing:** $p<.001$, η_p^2=.36; **Age*Housing**: ns. **Nucleus Accumbens: p250GAP: Age:** *ns*. **Housing:** $p<.05$, η_p^2=.14, SI>SH (p=.07); **Age*Housing**: *ns*. **GluA1: Age:** $p<.001$, η_p^2=.23. **Housing:** *ns*; **Age*Housing**: ns. **GluA2: Age:** *ns*. **Housing:** $p<.05$, η_p^2=.20, SI>SH ($p<.01$); **Age*Housing**: ns.

Main findings:

- Expression of BDNF and DNMT3A increased in vHPC of aged animals as compared with Adult animals. Furthermore, SI animals showed a marginal increase in the expression of GluA1 subunit.
- In the dHPC, SI upregulated the expression of p250GAP, GluA1, GluA2, and NR2B and downregulated the expression of Cfl1.
- In the NAcc, SI increased the expression of p250GAP and the GluA2 subunit. Adult animals showed higher levels of the GluA1 subunit than age counterparts. This effect was descriptively higher in adult SI animals.
- In all brain regions, SI increased the expression of at least one of the glutamate receptor subunits, an effect that was not age-dependent.

Summary and conclusion

- In agreement with our previous results, aged animals outperformed adult rats on OF habituation and spatial memory. Such an age effect was more pronounced in SI and SH rats on OF defensive responses. Between EE groups, the age differences were the smallest, except for total grooming and sequential grooming with variations which were particularly higher in adult EE animals. In age rats, in contrast, EE rather increased resting time instead of grooming behavior. In the spatial memory in the BM, aged rats benefited more from the EE experience than adults rats.
- At the brain level, aged animals showed a mixed pattern of gene expression, with some genes changing in a direction consistent with a scenario of reduced neural plasticity associated with age (i.e., ↑ p250GAP, ↑ DNMT3A, ↓ GluA1), but with other genes rather showing a pattern consistent with an increased neural plasticity (↑ BDNF in the vHPC; ↑ NR2B in the dHPC). Similarly, SI caused an upregulation of the subunits of the glutamatergic receptors in the dHPC, a change typically associated with increased neural plasticity. However, SI also increased the expression of p250GAP in the dHPC and the NAcc, which may be associated with a reduction in neural plasticity. EE caused no particular alterations in the expression of any of the studied genes, in despite of the behavioral changes it induced. Remarkably, SI induced no particular behavioral alterations at any age.
- Contrary to our expectations, aged animals outperformed adults in different behavioral domains but still were benefited from the EE experience. Remarkably, EE promoted an age-dependent shift in the strategy to cope stress, increasing the grooming in adults and the resting behaviors in aged individuals.
- None of the studied molecular targets accounted for the behavioral changes induced by the EE. However, the opposite pattern was found in SI animals: different molecular targets were up or down regulated despite the absence of behavioral alterations.

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Acknowledgments

Behavioral analysis (research assistants): **Rita Leandro, Paula Castro, Jimmy Chinchilla, Esteban Sánchez.** Financial support: **Project 837-B7-603 and 723-B9-197** through the **Vicerrectory of Research**, University of Costa Rica. Economic travel support: **Rectory**, University of Costa Rica; **Vicerrectory of Research**, University of Costa Rica.

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