Habituation and spatial memory in the context of emotional regulation: Behavioral and genetic mechanisms underlying context information-processing and de-arousal grooming

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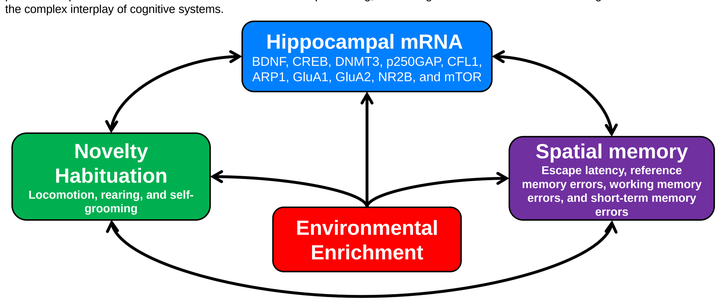
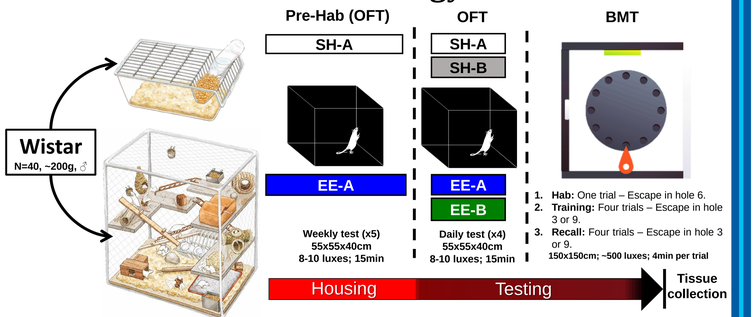
Introduction

Habituation is the ability to passively reduce a response after repeated or prolonged exposures to a particular stimulus. From a cognitive perspective, habituation is a basic, information-gating process that contributes to filter out irrelevant information in order to focus cognitive sources on a specific goal. In higher order capabilities such as spatial memory, a similar process occurs with the purpose of facilitate navigation towards the target place. In rodents, some forms of physical and social stimulation, like environmental enrichment (EE), potentiate both habituation and spatial memory. Here, we examined whether habituation capacity predicts spatial memory in the Barnes maze test (BMT). Male Wistar rats were kept for 30 days either on EE or on standard housing. During that time, half of the animals within each group were tested weekly in a 15-min open-field test (OFT) with the aim to evaluate long-term habituation. After the housing period, all rats were tested during four consecutive days in the OFT to assess short-term habituation. Afterwards, a three-day BMT protocol was used to evaluate several spatial and non-spatial memory parameters. To assess some brain mechanisms related with memory formation and brain plasticity, the hippocampal mRNA levels of BDNF, CREB, p250GAP, CFL1, DNMT3, ARP2, mTOR, GluA1, GluA2, and NR2B genes were determined. Evidence regarding the effects of EE on short-term and long-term OFT habituation and on BMT is be provided. Thus, we show the likely contribution of OFT behaviors, including certain types of grooming behavior, as predictors of spatial memory. Furthermore, we also show the association between gene expression and behavioral parameters. Because non-associative memory is observed in a plethora of species as a first-level mechanism of information processing, elucidating its functions could shed some light to better understand the complex interplay of cognitive systems.



Methodology



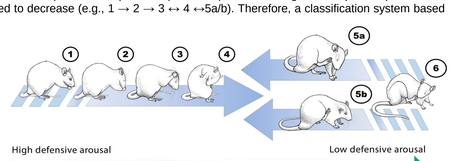
Notes: Pre-Hab: Animals under this condition were exposed to the OFT once a week during the 30-days housing period. **SH:** Standard housing; **EE:** Environmental enrichment; **A:** Group exposed to Pre-Hab; **B:** Group without Pre-Hab; **OFT:** Open-field test; **BMT**: Barnes Maze Test. EE cages were reconfigured twice a week.

Environmental enrichment: EE cages (120x70x100 cm) are custom-made steel boxes enclosed by metal grid, with one base floor and three additional aerial stainless-steel levels. All the objects and physical stimuli used in the cage were made of natural or semi-natural materials.

Behavioral analysis:

OFT: Locomotion: Automatically registered by Any-Maze®. **Rearing**: Manually scored by trained observers. Biped posture (free-standing or against the walls) elevated ≥ 45° from the floor. **Grooming:** Manually scored by trained observers. We have previously observed that short bouts in the head area are prompted to appear during the initial phases of exploration (i.e., 1-2-3), whereas long and complex sequences are displayed later on when exploratory activity has started to decrease (e.g., 1 → 2 → 3 ↔ 4 ↔5a/b). Therefore, a classification system based on those findings was developed.

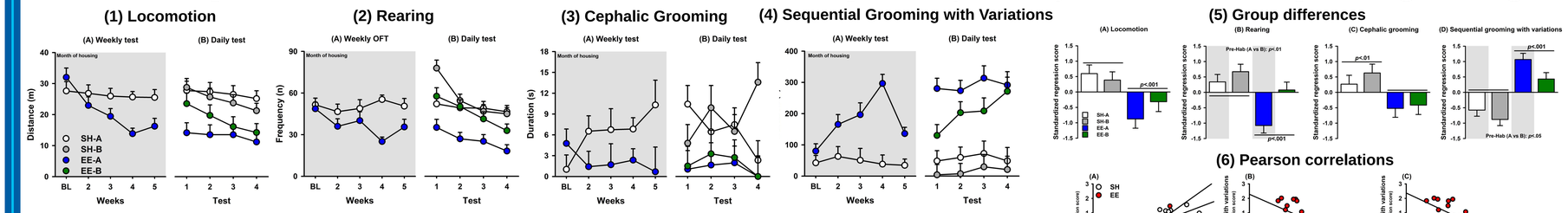
BMT: All variables were manually scored. **Escape latency:** The duration required by the animal to find the escape hole. **Reference memory errors:** Visit to a hole that was never associated with a escape. **Working memory errors:** Revisit the same hole within a ≤30s period. **Short-term memory errors:** Revisit the same hole within a >30s period. Once the animal put the four paws in the escape box, the hole was covered and the animal stayed there during 60s.



Analysis: Repeated measures ANOVA was used to compared temporal variables (i.e., repeated OFT tests, and trials in the BMT). Between-groups ANOVA was also used. Since no main effect of Pre-Hab was found in any of the variables measured in the BMT, a mixed ANOVA was carried out with Housing as between-group factor, and Trials as within-group factor. We performed a Principal Component Analysis (PCA) to generate one composite score of a latent construct of correlated indicators. In the OFT, a composite for (1) Locomotion (88% explained variance), (2) Rearing (75% explained variance), (3) Cephalic grooming (50% explained variance), and (4) Sequential grooming with variations (78% explained variance), was calculated with the total scores of each variable in the four one-day apart OFTs. In the BMT, a composite for (1) Latency of escape (71% explained variance), (2) Reference memory errors (45% explained variance), (3) Working-memory errors (56% explained variance), and (4) Short-term memory errors (60% explained variance), was calculated with the average scores of each variable in the three phases of testing (i.e., Habituation, Training, and Recall).

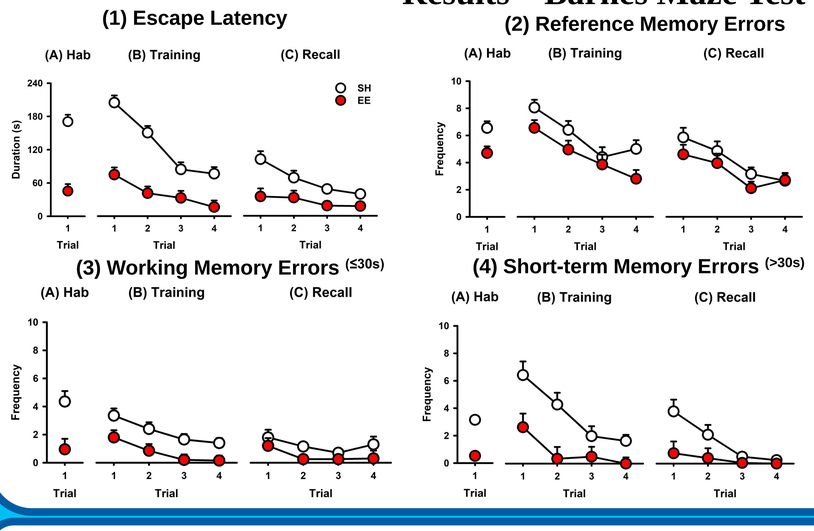
RNA quantification: One week after the last BMT trial, brains were dissected and the whole unilateral hippocampus was extracted and homogenized during 20s in 300µL of TRIzol. Then, the RNA was stored at -70°C for further processing. RNA was extracted according to manufacturer's specifications, and quantified 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.

Results – Open Field Test: EE enhanced the habituation of exploratory activity and increased sequential grooming



Locomotion: Fig. 1. A. Differences by OFT, $F(4,964)=13.19$, $p<.001$, $\eta^2=.45$, with OFT*Housing interaction, $F(4,64)=8.09$, $p<.001$, $\eta^2=.37$. **1.B.** Differences by OFT, $F(3,90)=12.59$, $p<.001$, $\eta^2=.30$, Housing, $F(1,30)=13,68$, $p<.001$, $\eta^2=.31$, with OFT*Pre.Hab interaction, $F(3,90)=3.78$, $p<.05$, $\eta^2=.11$. **Rearing: 2.A.** Differences by Housing, $F(1,16)=6.21$, $p<.05$, $\eta^2=.28$; with OFT*Housing interaction, $F(4,64)=3.59$, $p<.01$, $\eta^2=.18$. **2.B.** Differences by OFT, $F(3,105)=25.91$, $p<.001$, $\eta^2=.28$; Housing, $F(1,35)=16,67$, $p<.001$, $\eta^2=.32$, Pre.Hab, , $F(3,105)=10.06$, $p<.01$, $\eta^2=.23$, with OFT*Pre.Hab interaction, $F(3,105)=6.00$, $p<.001$, $\eta^2=.15$. **Cephalic grooming: 3.A.** No significant differences were found. **3.B.** Differences by Housing, $F(1,35)=10.17$, $p<.001$, $\eta^2=.23$. **Sequential grooming with variations: 4.A.** Differences by OFT, $F(1,16)=27.72$, $p<.001$, $\eta^2=.63$; Housing, $F(3,105)=25.91$, $p<.001$, $\eta^2=.28$; with OFT*Housing interaction, $F(4,64)=4.89$, $p<.01$, $\eta^2=.23$. **4.B.** Differences by Housing, $F(1,35)=54.45$, $p<.001$, $\eta^2=.61$; Pre.Hab, $F(1,35)=5.06$, $p<.05$, $\eta^2=.13$. **Group differences: 5.A.Locomotion,** Differences by Housing, $F(1,30)=13.83$, $p<.001$, $\eta^2=.32$; **5.B. Rearing.** Differences by Pre-Hab, $F(1,35)=9.11$, $p<.01$, $\eta^2=.21$; Housing, $F(1,35)=16,71$, $p<.001$, $\eta^2=.32$; **5.C. Cephalic grooming**. Differences by Housing, $F(1,35)=9,83$, $p<.01$, $\eta^2=.22$. 5.D. Sequential grooming with variations. Differences by Pre-Hab, $F(1,35)=5.45$, $p<.05$, $\eta^2=.13$, Housing, $F(1,35)=53.89$, $p<.001$, $\eta^2=.61$. **Note: BL:** Base-line OFT performed the first day of housing right before the animals were separated in groups. **Month of housing:** First month of exposure to the housing conditions.

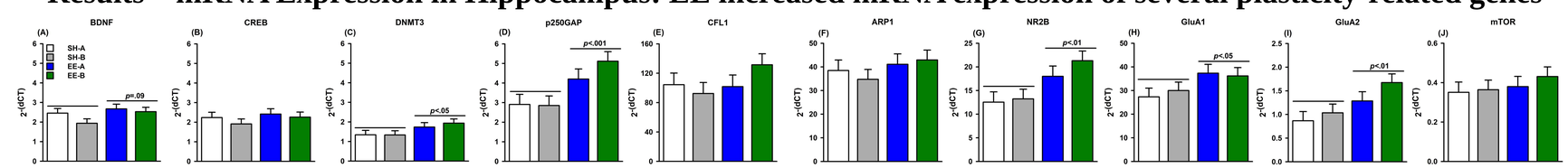
Results – Barnes Maze Test : EE increased spatial memory performance



Escape latency: Habituation - Fig.1.A. Differences by Housing, $F(1,36)=4.14$, $p<.001$, $\eta^2=.57$. **Training – Fig1.B.** Differences by Trial, $F(3,108)=33.03$, $p<.001$, $\eta^2=.48$. Housing, $F(1,36)=49.83$, $p<.001$, $\eta^2=.58$, with Trial*Housing interaction, $F(3,108)=6.71$, $p<.001$, $\eta^2=.16$. **Recall – Fig1.C.** Differences by Trial, $F(1.96,70.70)=7.33$, $p<.001$, $\eta^2=.17$, Housing, $F(1,36)=14.23$, $p<.001$, $\eta^2=.28$. **Reference memory errors: Habituation - Fig.2.A.** Differences by Housing, $F(1,36)=7.21$, $p<.05$, $\eta^2=.17$. **Training – Fig1.B.** Differences by Trial, $F(3,108)=12.35$, $p<.001$, $\eta^2=.25$. Housing, $F(1,36)=7.68$, $p<.01$, $\eta^2=.18$. **Recall – Fig1.C.** Differences by Trial, $F(2.35,84.76)=10.80$, $p<.001$, $\eta^2=.23$. **Working memory errors: Habituation - Fig.1.A.** Differences by Housing, $F(1,36)=23.86$, $p<.001$, $\eta^2=.40$. **Training – Fig1.B.** Differences by Trial, $F(1.97,70.81)=8.83$, $p<.001$, $\eta^2=.20$. Housing, $F(1,36)=13.90$, $p<.001$, $\eta^2=.28$. **Recall – Fig1.C.** No significant differences were found. **Short-term memory errors: Habituation - Fig.1.A.** Differences by Housing, $F(1,36)=15.04$, $p<.001$, $\eta^2=.35$. **Training – Fig1.B.** Differences by Trial, $F(2.19,79.02)=11.52$, $p<.001$, $\eta^2=.24$. Housing, $F(1,36)=14.89$, $p<.001$, $\eta^2=.29$. **Recall – Fig1.C.** Differences by Trial, $F(1.47,52.92)=5.92$, $p<.01$, $\eta^2=.14$. Housing, $F(1,36)=11.06$, $p<.01$, $\eta^2=.23$. No Pre-Hab differences were found in any variable.

Barnes Maze Test: Escape latency - Fig. 5.A. Differences by Housing, $F(1,36)=68.41$, $p<.001$, $\eta^2=.65$. **Reference memory errors – Fig. 5.B.** Differences by Housing, $F(1,36)=14.03$, $p<.001$, $\eta^2=.28$. **Working memory errors – Fig. 5.C.** Differences by Housing, $F(1,36)=16.02$, $p<.001$, $\eta^2=.38$. **Short-term memory errors – Fig. 5.D.** Differences by Housing, $F(1,36)=27.97$, $p<.001$, $\eta^2=.44$.

Results – mRNA Expression in Hippocampus: EE increased mRNA expression of several plasticity-related genes



mRNA Expression in the Hippocampus - BDNF Fig. A. Differences by Housing, $F(1,34)=3.04$, $p=.09$, $\eta^2=.08$. **CREB Fig. B.** No significant differences were found. **DNMT3 Fig. C.** Differences by Housing, $F(1,34)=5.21$, $p<.05$, $\eta^2=.13$. **p250GAP Fig. D**. Differences by Housing, $F(1,34)=12.57$, $p=.001$, $\eta^2=.27$. **CFL1 Fig. E.** No differences were found. **ARP1. Fig. F.** No differences were found**. NR2B Fig. G**. Differences by Housing, $F(1,34)=10.03$, $p<.01$, $\eta^2=.23$. **GluA1 Fig. H**. Differences by Housing, $F(1,34)=4.97$, $p<.05$, $\eta^2=.13$. **GluA2. Fig. I.** $F(1,34)=7.81$, $p<.01$, $\eta^2=.19$. **mTOR Fig. J.** No differences were found.

Summary

- •EE facilitated the habituation of exploratory and risk-assessment behaviors (i.e., locomotion and rearing), and caused a dissociated effect on grooming behavior: A reduced display of cephalic grooming contrasted with an increased time spent on sequential grooming with variations. Also, regardless the housing condition, the animals that were pre-habituated to the OFT spent less time rearing and more time displaying sequential grooming with variations. Finally, exploratory and risk-assessment behaviors were negatively associated with sequential grooming with variations in enriched, but not in SH rats. The enhanced cognitive abilities of enriched animals would explain the faster transition from exploratory activities to other self-directed behaviors observed in these animals. In particular, EE would have increased sequential grooming with variations as a compensatory strategy involved in the process of emotional de-arousal
- EE animals showed augmented spatial memory performance in all BMT variables. Enriched animals did not reduce the reference and working memory errors during the recall phase, as compared with SH animals. Escape latency was positively associated with reference and short-term memory errors in both groups. Nevertheless, only in SH animals working memory errors and escape latencies were positively associated, suggesting that other forms of long-term memory were favored by EE, which translated into a more efficient navigation to find the escape hole.
- EE increased the hippocampal mRNA expression of several genes involved in different forms of brain plasticity. It would be possible that EE-induced changes in the expression of NMDA (NR2B) and AMPA (GluA1 and GluA2) receptor sub-units underlie the enhanced memory performance observed these animals. Interestingly, the expression of p250GAP –a protein associated with inhibition of dendritic branching– was also increased after EE. Upregulation of p250GAP may be a mechanism for neuropil remodeling to promote synaptic efficiency after long-term stimulation. Such a synaptic reorganization may involve dendritic spine reshaping, a process that is known to be regulated by p250GAP as well. However, the mRNA levels of proteins associated with dendritic branching, such as mTOR, CFL1, and ARP1 were unaffected by EE, suggesting that remodeling of actin cytoskeleton –including dendritic morphogenesis or pruning– took place at earlier stages of hippocampal plasticity. Alternatively, upregulation of p250GAP promoted by EE may have recruited other molecular mechanisms of plasticity independent from mTOR, CFL1, and ARP1.
- At a behavioral level, correlation analysis (data not shown) revealed multiple associations within- and between-tests, suggesting a common learning and memory domain between OFT and BMT, which was differentially affected by housing conditions. At a molecular level, genes were strongly correlated among them but with a different association pattern within each group. Such particular Gene-Behavior correlations suggest that between-groups differences in novelty habituation and spatial memory would be related with those molecular targets.

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