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Research report

Altered consolidation of extinction-like inhibitory learning in genotype-specific dysfunctional coping fostered by chronic stress in mice

P. Campus[a,b,1], M. Maiolati[a], C. Orsini[a,b], S. Cabib[a,b,*]

[a] Department of Psychology, Center D. Bovet, Sapienza University of Rome, Rome, Italy
[b] Fondazione Santa Lucia, IRCCS, Rome, Italy

HIGHLIGHTS

- Food restricted C57BL/6J mice show enhanced and DBA/2J mice show reduced infralimbic c-fos induction by acute stress.
- The Infralimbic Cortex is involved in consolidation of an acquired coping strategy in both mouse strains.
- Food restricted C57BL/6J mice show enhanced and DBA/2J mice show reduced retention of escape extinction.

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ABSTRACT

Genetic and stress-related factors interact to foster mental disorders, possibly through dysfunctional learning. In a previous study we reported that a temporary experience of reduced food availability increases forced swim (FS)-induced helplessness tested 14 days after a first experience in mice of the standard inbred C57BL/6(B6) strain but reduces it in mice of the genetically unrelated DBA/2J (D2) strain. Because persistence of FS-induced helplessness influences adaptive coping with stress challenge and involve learning processes the present study tested whether the behavioral effects of restricted feeding involved altered consolidation of FS-related learning.

First, we demonstrated that restricted feeding does not influence behavior expressed on the first FS experience, supporting a specific effect on persistence rather then development of helplessness. Second, we found that FS-induced c-fos expression in the infralimbic cortex (IL) was selectively enhanced in food-restricted (FR) B6 mice and reduced in FR D2 mice, supporting opposite alterations of consolidation processes involving this brain area. Third, we demonstrated that immediate post-FS inactivation of IL prevents 24 h retention of acquired helplessness by continuously free-fed mice of both strains, indicating the requirement of a functioning IL for consolidation of FS-related learning in either mouse strain. Finally, in line with the known role of IL in consolidation of extinction memories, we found that restricted feeding selectively facilitated 24 h retention of an acquired extinction in B6 mice whereas impairing it in D2 mice.

These findings support the conclusion that an experience of reduced food availability strain-specifically affects persistence of newly acquired passive coping strategies by altering consolidation of extinction-like inhibitory learning.

1. Introduction

Increasing evidence supports a main role of dysfunctional learning [1–6] and of life-long interactions between genetic liability and adverse experiences [7–10] in the development of psychopathology. Therefore, the study of genotype-dependent effects of adverse experiences on learning mechanisms can advance the understanding of pathogenic processes.

A previous study from this laboratory found that a temporary experience of reduced food availability, a common adverse condition in natural settings, enhances Forced Swim (FS)-induced helplessness expressed 14 days after a first FS experience in mice of the standard inbred C57BL/6J (B6) strain, but reduces it in mice of the genetically unrelated inbred DBA/2J (D2) strain [11]. These find-

* Corresponding author at: Department of Psychology, Sapienza University of Rome, via dei Marsi 78, 00185 Rome, Italy.
E-mail address: simona.cabib@uniroma1.it (S. Cabib).
[1] Present address: Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA.

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ings suggest genotype-specific effects of the adverse experience on adaptive learning because persistence of FS-induced helplessness involves long-term memory [12–16] of an adaptive coping strategy [16–20].

Indeed, when first introduced to the FS apparatus rodents express both active (swimming and struggling to climb the walls of the water tank) and passive (immobility) coping responses. In the first minutes of experience, active coping responses predominate but they decrease overtime while immobility episodes increase in number and duration [16,17,20]. These behavioral changes indicate development of a passive coping strategy, generally known as helplessness, in a situation that is appraised as incontrollable/inescapable [18–20]. Helplessness is consolidated as long-term memory and on subsequent FS experiences, animals immediately express high levels of immobility and low levels of active responses, thus preventing useless and risky waste of energies [16,19–21]. Because alterations of these mechanisms disrupt adaptive stress coping and dysfunctional stress management is a marker of psychopathology [22], alterations of FS-induced behavior could model psychopathology in rodents. Finally, B6 and D2 strains are useful models for the study of genotype-dependent effects of adverse experiences on learning processes. Indeed, the two mouse strains develop different or opposite stress-induced neural phenotypes [23–29] and utilize different brain memory systems to learn under stress [19].

These considerations support the relevance of investigating mechanisms involved in the strain-dependent effects of restricted feeding on FS-induced helplessness. The following experiments aimed at evaluating whether altered consolidation of FS-related learning is among these mechanisms.

Although changes in helplessness expressed by mice re-exposed to FS 14 days after a first experience [11] suggest altered persistence of the acquired coping strategy, data collected in those previous experiments [11] did not rule out an effect of restricted feeding on the initial behavioral response to FS or on the acquisition of helplessness. Therefore, in a first set of experiments we tested the effects of restricted feeding on behavior expressed during the first FS experience.

A second set of experiments evaluated the effects of restricted feeding on FS-induced c-fos expression in different brain areas because increased c-fos expression represents a good index of plasticity associated with memory consolidation. Indeed, a rapid and transient expression of c-fos is associated with the initial steps of memory consolidation in different learning tasks and reduced/impaired c-fos expression during training prevents consolidation of persistent memories [30–36]. Moreover, c-fos expression has been causally linked to epigenetic modulation of long-term memory [21,30,37].

The subsequent two sets of experiments were planned to test the hypothesis, derived by findings of the previous sets of experiments, that a dysfunctional infralimbic cortex (IL) mediates opposite alteration of consolidation processes in FR mice of the two strains. To test this hypothesis, we first evaluated whether a functioning IL is indeed necessary for consolidation of long-term memory of FS-related learning in mice of both strains. In a final set of experiments we tested whether FR experience strain-specifically influences retention of extinction learning because of the main role of IL in extinction development, consolidation and retrieval [38].

2. Materials and methods

2.1. Animals and housing

Male mice of the inbred DBA/2J (D2) and C57BL/6J (B6) strains (Charles River, Como, Italy) were purchased at 6 weeks of age and housed in groups of four in standard breeding cages with food and water at libitum on a 12-h dark/light cycle (lights on between 07:00 h and 19:00 h) at a temperature of 22 ± 1 °C. When animals reached 7 weeks of age they were individually housed and assigned to experimental group (food-restricted: FR) or to control group (continuously free-fed: FF) [39–41]. Mice were all individually housed to avoid aggression in the FR groups, reduce manipulation-induced stress during tissue collection in the control groups, and reduce differences between cannulae-implanted and non-implanted animals.

FR mice received food once daily in a quantity adjusted to reach 85% of the initial body weight within the first 3 days and maintain it thereafter. FF mice received food once daily in a quantity adjusted to exceed daily consumption (15 g). On the evening of the 12th day mice from both groups were given food ad libitum and left undisturbed for 48 h before behavioral testing or tissue collection. Experiments were conducted according to the Italian national law on the use of animals for research (DL 116/92).

2.2. Experiment 1

The first set of experiments tested the effects of FR on acquisition of FS-induced immobility in the two mouse strains. A group of FF (n = 6) and a group of FR (n = 6) mice from each strain was used.

2.2.1. Behavioral response to forced swim

Apparatus and procedures were previously described [15]. Briefly, Individual mice were gently laid in the water contained in a glass cylinder where they were left for 10 min. Behavior was registered by a digital video camera located frontally to the apparatus and connected to a computer located within a different room. Duration (s) of struggling to climb out, swimming, and immobility (absence of all movements not required to float), was scored on videotapes by a trained experimenter unaware of the experimental groups by the aid of EthoVision (Noldus Netherlands).

2.2.2. Statistical analyses

Statistical analyses were performed on data collected during 2 blocks of 5 min each with a two-way ANOVA for repeated measures (between factor: feeding, two levels = FF, FR; within factor: 5 min blocks, 2 levels). The main effect of the repeated measure was then tested within each group (FF,FR).

2.3. Experiment 2

The second set of experiments tested the effects of restricted feeding on c-fos expression promoted by the 10 min FS experience in different brain areas of the two mouse strains.

A total of 24 mice from each strain (12 FF, 12 FR) were used for these experiments. One half of the animals from each group was sacrificed immediately after removal from their home cages (naive). The other half was sacrificed 50 min after a first experience of forced swim.

2.3.1. Forced swim stress

Apparatus was the same as described above. Individual mice were gently laid in the water contained in a glass cylinder and left for 10 min. Following the experience mice were left undisturbed within their home cages for 50 min before being killed for tissue collection. Mice not exposed to FS (Naive) were killed immediately upon removal from their home cages

2.3.2. Immunohistochemistry

All mice were killed by decapitation and after removal, brains were immersed in chilled 10% neutral buffered formalin and stored overnight and then cryoprotected in 30% sucrose solution at 4 °C