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ENRICHED ENVIRONMENT EFFECTS ON REMOTE OBJECT RECOGNITION MEMORY

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Abstract—Since Ebbinghaus' classical work on oblivion and saving effects, we know that declarative memories may become at first spontaneously irretrievable and only subsequently completely extinguished. Recently, this time-dependent path toward memory-trace loss has been shown to correlate with different patterns of brain activation. Environmental enrichment (EE) enhances learning and memory and affects system memory consolidation. However, there is no evidence on whether and how EE could affect the time-dependent path toward oblivion. We used Object Recognition Test (ORT) to assess in adult mice put in EE for 40 days (EE mice) or left in standard condition (SC mice) memory retrieval of the familiar objects 9 and 21 days after learning with or without a brief retraining performed the day before. We found that SC mice show preferential exploration of new object at day 9 only with retraining, while EE mice do it even without. At day 21 SC mice do not show preferential exploration of novel object, irrespective of the retraining, while EE mice are still capable to benefit from retraining, even if they were not able to spontaneously recover the trace. Analysis of c-fos expression 20 days after learning shows a different pattern of active brain areas in response to the retraining session in EE and SC mice, with SC mice recruiting the same brain network as naïve SC or EE mice following de novo learning. This suggests that EE promotes formation of longer lasting object recognition memory, allowing a longer time window during which saving is present.

Keywords: enriched environment, object recognition, long-term memory, saving effect, brain activation.

INTRODUCTION

The life of a memory trace is quite complex, and it crosses many steps from the encoding of information to its consolidation in a long lasting trace. We know that the process leading to the formation of a long-lasting declarative memory involves different molecular mechanisms and progressive recruitment of brain areas in what is known as system consolidation (Squire and Alvarez, 1995; Frankland and Bontempi, 2005; Romero-Granados et al., 2010; Bonaccorsi et al., 2013). Forgetting, as assessed by absence of spontaneous recall, can be due to at least two reasons: the memory trace is still present, stored in the brain, but inaccessible to recall; or the memory is no longer stored in the brain (Mirman and Britt, 2013). The first to experimentally study oblivion was Herman Ebbinghaus at the end of 1800. Using lists of non-sense words, he calculated the number of items that he progressively forgot with time, drawing the ''oblivion curve". He also developed the concept of ''saving", meaning the facilitation to re-learn non-novel items thanks to the past learning, suggesting that, before becoming completely extinguished, a memory trace crosses a stage during which the effects of learning are not completely lost, but the trace is still present, although inaccessible to spontaneous recall (Ebbinghaus, 1885).

Recently, Romero-Granados and coworkers, using Object Recognition Test (ORT), proposed a model in which a declarative memory trace crosses, with time after learning, two stages: a first stage in which it is apparently forgotten, in that it is not spontaneously recoverable, but the effects of learning are not completely lost, in that the long-term memory of the familiar object can be recovered after a brief retraining (Romero-Granados et al., 2010); a second stage in which the trace is unrecoverable even following brief retraining. These two different states of an apparently lost memory, still recoverable following retraining and unrecoverable, correlate with different patterns of brain activation and of plasticity factors expression in specific areas. The model that emerges from these data suggest that following consolidation, a memory trace can be easily recalled within a certain time period, then it is ''hidden", seemingly appearing extinguished because not available to free recall, but still available to ''assisted" recall and finally becoming no longer retrievable, suggesting total loss of the trace. It is not known whether this time course toward oblivion is predetermined or can be affected by manipulations of the environmental experience, such as that provided by EE, which is known to profoundly affect brain plasticity and to enhance learning and memory (Sale et al., 2014).

Many papers have indeed underlined the beneficial effects of EE on memory acquisition and on recovery

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Abbreviations: ANOVA, analysis of variance; EE, environmental enrichment; ORT, Object Recognition Test; PFA, paraformaldehyde; SS, standard condition.

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from cognitive deficits, in aged animals or in animal models of neurodegenerative diseases (Van Praag et al., 2000; Duffy et al., 2001; Berardi et al., 2007; Pizzorusso et al., 2007; Bekinschtein et al., 2011; Leger et al., 2012; Sale et al., 2014); however, whether EE could affect the time-dependent path toward oblivion and the "saving" effect is still not known.

The aim of our study is to verify first whether EE allows to form an object recognition memory trace recoverable for a longer time, either under conditions of spontaneous recall or under conditions of assisted recall, distinguishing therefore between different types of oblivion (trace loss and recovery failure) and second to investigate the possible neural substrates for this EE effect. We found that EE promotes formation of longer lasting object recognition memory with respect to SC, slowing down the path toward memory-trace loss and prolonging the time window during which saving is present.

This correlates with a different pattern of active brain areas in response to the retraining session in EE and SC mice.

EXPERIMENTAL PROCEDURES

Animals and rearing conditions

A total of 165 adult male and female C57BL/6 mice were used in this study (n = 82 males, n = 83 females). All procedures were approved by the Italian Ministry of Health. Animals were housed in an animal room with a 12 h/12-h light/dark cycle, with food and water available *ad libitum*, and experiments were performed during the light phase (Berardi et al., 2007). At 2 months of age, animals were assigned to one of the following rearing conditions for 40 days: Environmental Enrichment (EE: n = 84, males n = 42, females n = 42) or standard condition (SC: n = 81, males n = 40, females n = 41). SC rearing consisted of 26 × 18 × 18-cm cages housing 3–5 animals; EE rearing condition was achieved using large cages (44 × 62 × 28 cm) housing 6–10 animals, containing several food hoppers, one running wheel for voluntary physical exercise, and differently shaped objects (tunnels, toys, shelters, stairs) that were repositioned twice a week and completely substituted with others once a week (Berardi et al., 2007).

Experiments on EE mice begun after 40 days in EE; after the beginning of experiments, no more novel stimuli were inserted in the cages, to avoid interferences with learned objects. The position of objects inside the cages was however changed twice a week to maintain environmental stimulation.

Apparatus

We run the ORT in a Y-apparatus (Bartko et al., 2010; Leger et al., 2012) with high, homogenous white walls constructed from Perspex to prevent the mouse from looking out into the room, thereby maximizing attention to the stimuli. One arm was used as the start arm, and had a sliding door to allow access to the arena; the other two arms were used to display the objects. All walls were 30 cm high; the start arm was 26 cm long with the sliding door placed at 13 cm from the arm end. The lateral arms were 18 cm long and all arms were 10 cm wide. The apparatus was placed in a silent room within a box with white walls and ceiling; a video camera was mounted above the apparatus and all trials were recorded with the Ethovision software (Noldus 9.0).

Experimental design and behavioral procedures

The protocol for behavioral tests is outlined in Fig. 1. On the first day (Day 0) mice were habituated to the Y-shape arena for 20 min. The learning session (Sample) was performed 24 h later (Day 1) allowing the mice to explore for 15 min two identical objects, each placed at the end of the short arms. Exploration time was taken when mice approached the objects with muzzle and paws. The experimenter measuring exploration time was blind to rearing condition and treatment. The test phase was performed the day after the learning session (Day 2) for all animals, except the naïve group described later, to be sure that learning occurred, and then either following 9-day or 21-day interval (Day 9/Day 21), depending on the experimental condition, changing one of the two familiar objects (those explored during the sample phase) with a novel one and the other familiar object with an identical one, and allowing the mice to explore them for 5 min.

A total of 42 EE and 42 SC animals performed the test phase at day 9 or 21 (groups 9 days EE, n = 21, 10 males, 11 females; 9 days SC, n = 23, 11 males, 12 females; 21 days EE, n = 21, 10 males, 11 females; 21 days SC, n = 19, 10 males, 9 females). Some animals performed the test at day 9 or 21 following a brief retraining session at day 8 or 20 (9 days EE-RET, n = 10, 5 males and 5 females; 9 days SC-RET n = 12, 6 males and 6 females; 21 days EE-RET n = 10, 5 males and 5 females; 21 days SC-RET n = 12, 6 males and 6 females) while other animals performed the test without a preceding retraining session (9 days EE-NO RET n = 11, 5 males and 6 females; 9 days SC-NO RET n = 11, 5 males and 6 females; 21 days EE-NO RET n = 11, 6 males and 5 females; 21 days SC-NO RET n = 7, 4 males and 3 females). The retraining session consisted in a brief (3 min) exposure to the familiar objects.

To test for a saving effect, the time length of the brief retraining session should not able to give rise per se to a new long lasting memory. We controlled for this subjecting a separate group of animals, 27 EE and 24 SC, to the habituation phase on Day 0, to a learning phase of 3 min (EE n = 13, 6 males and 7 females; SC, n = 11, 5 males and 6 females) or 15 min (EE n = 14, 7 males and 7 females; SC, n = 13, 6 males and 7 females) at Day 1 and to the test phase at Day 2 (see protocol in Fig. 1).

Arena and objects were cleaned up between trials to stop the build-up of olfactory cues. Objects were simple 3D objects derived from everyday living, and their dimensions were 10–20-cm height and 6–8-cm width. To avoid possible spontaneous preferences for one of the objects, the choice of the new and old object and

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