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Either the dorsal hippocampus or the dorsolateral striatum is selectively involved in consolidation of forced swim-induced immobility depending on genetic background

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ABSTRACT

Healthy subjects differ in the memory system they engage to learn dual-solution tasks. Both genotype and stress experience could contribute to this phenotypic variability. The present experiments tested whether the hippocampus and the dorsal striatum, the core nodes of two different memory systems, are differently involved in 24 h retention of a stress-associated memory in two genetically unrelated inbred strains of mice.

Mice from both the C57BL/6J and the DBA/2J inbred strains showed progressive increase of immobility during 10 min exposure to forced swim (FS) and retrieved the acquired levels of immobility when tested 24 h later. The pattern of c-fos immunostaining promoted by FS revealed activation of a large number of brain areas in both strains, including CA1 and CA3 fields of the hippocampus. However, only DBA/2J mice showed activation of the dorsolateral striatum (DLS). In addition, FS induced a positive correlation between c-fos expression in the amygdala and CA1 and CA3 in C57BL/6J mice whereas it induced a positive correlation between c-fos expression in the amygdala and DLS in DBA/2J mice. Finally, temporary post-training inactivation of the dorsal hippocampus, by local infusion of lidocaine, prevented 24 h retention of immobility in C57BL/6J mice only, whereas inactivation of the DLS prevented retention in DBA/2J mice only.

These findings support the view that genetic factors can determine whether the dorsal hippocampus or the DLS are selectively engaged to consolidate stress-related memory.

1. Introduction

Learning and memory can be supported by multiple memory systems that process information simultaneously and in parallel. A group of recent findings revealed that some individuals consistently engage a hippocampus-centered memory system whereas others engage a dorsal striatum-centered one to learn double-solution tasks. Indeed, healthy participants were shown to use either place or response learning strategies selectively and preference for a specific strategy was associated with specific functional and structural neurophenotypes involving the hippocampus and dorsal striatum (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; Marchette, Bakker, & Shelton, 2011).

There is some evidence that genetic factors contribute to this phenotypic variability. Indeed, results of a recent study on the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) revealed that 'Met' carriers use a response strategy more frequently than individuals homozygous for the 'Val' allele (Banner, Bhat, Etchamendy, Joober, & Bohbot, 2011). On the other hand, both human and rodent studies indicate that stressed organisms preferentially engage striatum-dependent learning strategies (Dias-Ferreira et al., 2009; Packard, 2009; Schwabe & Wolf, 2013), supporting a strong contribution of stress to development and expression of individual learning styles.

Studies in animal models can directly test the involvement of a specific memory system in the different steps that lead to development of long-term memory. Indeed, temporary inactivation of selected brain areas performed before or immediately after training have been shown to interfere with acquisition and consolidation of specific memories selectively, whereas inactivation before test have been shown to selectively interfere with retrieval of the acquired memory (Coleman-Mesches & McGaugh, 1995; Cox,

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Czerniawski, Ree, & Otto, 2013; Daumas, Halley, Frances, & Lassalle, 2005; Oliveira, Hawk, Abel, & Havekes, 2010; Vazdarjanova & McGaugh, 1999).

In addition, research in animal model allows manipulation of stress and genetic variables in controlled experimental settings. Learning styles and associated neurophenotypes seem to be polygenic-many genes contribute, each to a very small extent (Plomin, Haworth, & Davis, 2009) because most people use both place and response learning whereas only a few show a true learning style (Marchette et al., 2011). Neurobiological determinants of polygenic phenotypes are best targeted by studies in inbred mouse strains that facilitate the unbiased discovery of biological and genetic correlation. Then, recombinant inbred strains (RI) can be used to sort out genetic control of the phenotypic variation (Peirce, Lu, Gu, Silver, & Williams, 2004).

Mice of the inbred DBA/2J (D2) and C57BL/6J (B6) strains represent a useful animal model for studies on neurobiological bases of individual learning strategies. Indeed, D2 mice have been reported not to use hippocampus-dependent strategies in various learning tasks, in sharp contrast with the bias toward the use of these strategies by mice of the genetically unrelated B6 inbred strain (Ammassari-Teule, Passino, Restivo, & de Marsanich, 2000; Baarendse et al., 2008; Bovet, Bovet-Nitti, & Oliverio, 1969; Restivo, Roman, Ammassari-Teule, & Marchetti, 2006). Moreover, recent results indicate that D2 mice engage hippocampus-dependent strategies when trained under very low stress levels (Youn et al., 2012), suggesting the hypothesis that D2 mice are more susceptible than B6 to shift to striatal learning strategies under stress.

However, there is still no evidence that D2 mice engage the dorsal striatum to learn a task that B6 mice learn through hippocampus-dependent strategies. Thus, the present experiments tested the hypothesis that D2 mice engage the dorsal striatum whereas B6 mice engage the hippocampus to develop a long-term stress-related memory. To this aim, we induced immobility in B6 and D2 mice by ten minutes exposure to forced swim (FS), a widely exploited stress procedure known to engage learning processes (Chandramohan, Droste, Arthur, & Reul, 2008; De Pablo, Parra, Segovia, & Guillamon, 1989; Korte, 2001; Mitchell & Meaney, 1991; West, 1990), evaluated retention of the response in a five minutes test performed 24 h later (FSt), mapped expression of the early gene c-fos induced by FS, and tested the involvement of the two brain areas on 24 retention of acquired immobility by temporary post-training inactivation.

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 experiments started one week later. We adopted this housing protocol for all the groups used in the experiments because group-housed mice would groom each other to the point of removing guide cannulae.

All experiments were conducted according to the Italian national law (DL 116/92) on the use of animals for research.

2.2. Forced-swimming procedure

The apparatus consisted of a glass cylinder (40 cm height, 18 cm diameter) containing 30 cm of water at 24 ± 2 °C. Mice were exposed to the apparatus for 10 min (Training session: FS) then removed from cylinder and returned to their home cage. 24 h later mice were re-exposed for 5 min to apparatus (test session: FSt). The behavior exhibited by each animal in the course of FS and FSt was videotaped using a remote-controlled video camera placed frontally to cylinder and videotapes were scored by a trained experimenter blind to housing condition by the aid of EthoVision (Noldus Netherlands). Animals used for immunohistochemical analysis were killed 50 min after FS.

2.3. Immunostaining and image analyses

Immunohistochemical analyses were performed, for all tissue samples, in two different batches: one for each strain. For each strain c-fos expression was evaluated in mice exposed to FS ($n = 6$) and in unhandled mice just removed from their home cages (Naïve; $n = 6$).

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 for 48 h (Colelli, Fiorenza, Conversi, Orsini, & Cabib, 2010; Conversi, Bonito-Oliva, Orsini, & Cabib, 2006). Frozen coronal sections (40 μm thickness) were cut through whole brain with a sliding microtome and then immunolabelled with immunoperoxidase method as previously described (Colelli et al., 2010; Conversi et al., 2006). Rabbit anti-c-fos (1/20,000; Oncogene Sciences) was used as primary antibody and secondary immunodetection was performed with a biotinylated antibody (1:1000 goat anti-rabbit, Vector Laboratories Inc., Burlingame, CA, USA). Peroxidase labeling was obtained by standard avidin–biotin procedure (Vectastain ABC elite kit, Vector Laboratories, diluted 1:500) and chromogenic reaction was developed by incubating sections with metal-enhanced DAB (Vector Laboratories). Two batches, each involving all experimental groups, sampled areas, and hemispheres of all mice from one strain, were prepared.

Sections were analyzed using a Nikon Eclipse 80i microscope equipped with a Nikon DS-5M CCD camera as previously described (Colelli et al., 2010; Conversi et al., 2006). Specimens were subjected to quantitative image analysis using the public domain image analysis software IMAGEJ 1.38g for Linux (Abramoff, Magelhaes, & Ram, 2004). For quantification, striatum was divided into four compartments: Dorsomedial (DMS), Dorsolateral (DLS); the Nucleus Accumbens in Core (NAc Co) and Shell (NAc Sh); the hippocampus in CA1, CA3 and Dentate Gyrus (DGY) fields; the

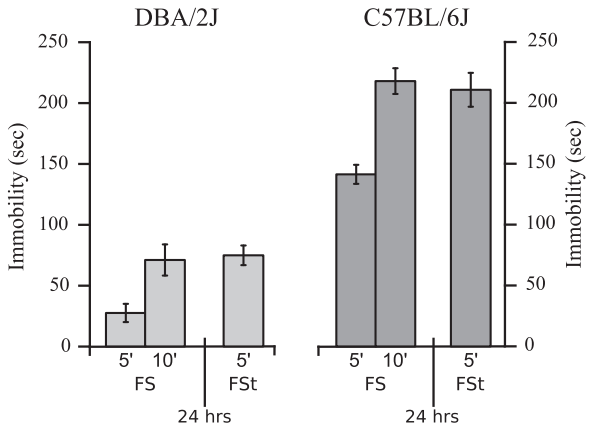

Fig. 1. Acquisition and 24 h retention of immobility (mean sec + SEM) by mice of the inbred DBA/2J and C57BL/6J strains. Mice from both strains showed a significant increase of the immobility in the course of the initial exposure to the stressor (FS) and retrieval of the acquired response 24 h later (FSt). See results for statistical details.