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Maintenance of long-term memory storage is dependent on late posttraining Egr-1 expression

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ABSTRACT

Expression of immediate-early genes, like Egr-1, has been shown to be induced by activity-dependent synaptic plasticity or behavioral training and is widely thought to play an important role in long-term memory (LTM) formation. However, little is known about the role of Egr-1 in the maintenance of memory storage. Here we show that dorsal hippocampal Egr-1 protein expression is upregulated between 12 and 24 h after strong inhibitory avoidance (IA) training in rats. Local infusion of antisense oligodeoxynucleotide (ASO) to specifically knockdown Egr-1 in the dorsal hippocampus 8 h posttraining impairs LTM tested 7 days, but not 1 day after training, indicating that a delayed learning-associated expression of Egr-1 is necessary for the persistence of LTM storage. In addition, we show that consolidation of the IA memory is accompanied by an increase in Egr-1 protein levels 3 h, but not immediately or 1 h after training. Local infusion of *egr-1* ASO 30 min before training in the dorsal hippocampus persistently hinders memory formation measured 1 and 7 days after IA training, indicating the crucial role of Egr-1 in memory formation. Our findings demonstrate that there are at least two waves of Egr-1 expression in the dorsal hippocampus after IA training, an early wave which is involved in IA LTM formation, and a lasting late wave that peaks around 12–24 h after a strong training protocol which is specifically involved in the maintenance of LTM storage.

1. Introduction

Early Growth Response-1 (Egr-1), also known as Zif-268 or Zenk, is a member of the zinc finger family of transcriptional factors induced by stress or injury, differentiation factors, and a variety of extracellular signals including neurotransmitters, peptides and growth factors (reviewed in Davis, Bozon, and Laroche (2003), Herdegen and Leah (1998) and O'Donovan et al. (1999)). Egr-1 regulates the expression of a number of late-response genes involved in growth control, survival and in plasticity-related processes in the brain (Bozon et al., 2003; Maddox, Monsey, & Schafe, 2011; Sukhatme et al., 1988; Williams et al., 2000). Egr-1 is rapidly induced by behavioral training in the amygdala (Rosen, Fanselow, Young, Sitcoske, & Maren, 1998) and hippocampus (Guzowski, Setlow, Wagner, & McGaugh, 2001; Miyashita, Kameyama, Hasegawa, & Fukushima, 1998; Nikolaev, Kaminska, Tischmeyer, Matthies, & Kaczmarek, 1992).

Egr-1 has an important role in learning and memory. Deletion of Egr-1 leads to a reduction of hippocampal late-long-term potentiation (L-LTP) as well as a LTM impairing for several tasks including spatial navigation in the Morris water maze, conditioned taste aversion, social transmission of food preference and object recognition (Jones et al., 2001). In addition, Egr-1 mutant mice are unable to consolidate information about the spatial location or the features of objects (Bozon et al., 2003). By using *egr-1* antisense oligonucleotides (ASO) infused into the amygdala, Malkani, Wallace, Donley, and Rosen (2004) found impaired expression of fear conditioning. Furthermore, Yang et al. (2012) infused *egr-1* ASO in the hippocampus and found an impairment in spatial memory consolidation and also the infusion before retrieval impaired reconsolidation of contextual fear conditioning memory (Lee, Everitt, & Thomas, 2004).

Despite the general consensus that Egr-1 participates in the mechanisms involved in memory consolidation and reconsolidation, there is no information concerning the role of Egr-1 in the mechanisms involved in the persistence of LTM storage. In a series of previous experiments carried out by our group we demonstrated a novel BDNF- and protein synthesis-dependent late consolidation phase in the dorsal hippocampus important for the persistence of

Abbreviations: LTM, long-term memory; IA, inhibitory avoidance; ASO, antisense oligodeoxynucleotide; MSO, missense oligodeoxynucleotide.

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memory storage (Bekinschtein et al., 2007). We also found that this phase is accompanied by an increase in Egr-1 protein expression 24 h after training. Therefore, the present study was designed to determine whether or not this late wave of Egr-1 expression in the dorsal hippocampus is required for maintaining the memory trace of a one-trial IA LTM in rats.

2. Material and methods

2.1. Subjects

Male Wistar rats (2.5 months/220–250 g) from our own breeding colony were used. Animals were housed five to a cage at 23 °C, with water and food *ad libitum*, under a 12 h light/dark cycle (lights on at 7:00 a.m.). The procedures followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the University of Buenos Aires.

2.2. Inhibitory avoidance training and testing

Animals were trained in a one-trial step-down inhibitory avoidance task (IA) as previously described (Bekinschtein et al., 2007). Briefly, the apparatus was a 50 × 25 × 25 cm acrylic box with a 5 cm high, 7 cm wide, and 25 cm long platform on the left end of a series of stainless steel bars that made up the floor of the box. For training, animals were gently placed on the platform; as they stepped down to the grid they received either a 3 s, 0.7 mA scrambled foot-shock (strong training) or a 3 s, 0,3 mA scrambled foot-shock (weak training). Rats were tested for retention 1 or 7 days after training. All animals were tested only once. In the test sessions the foot-shock was omitted. Significant differences on latency to step down between training and test sessions were

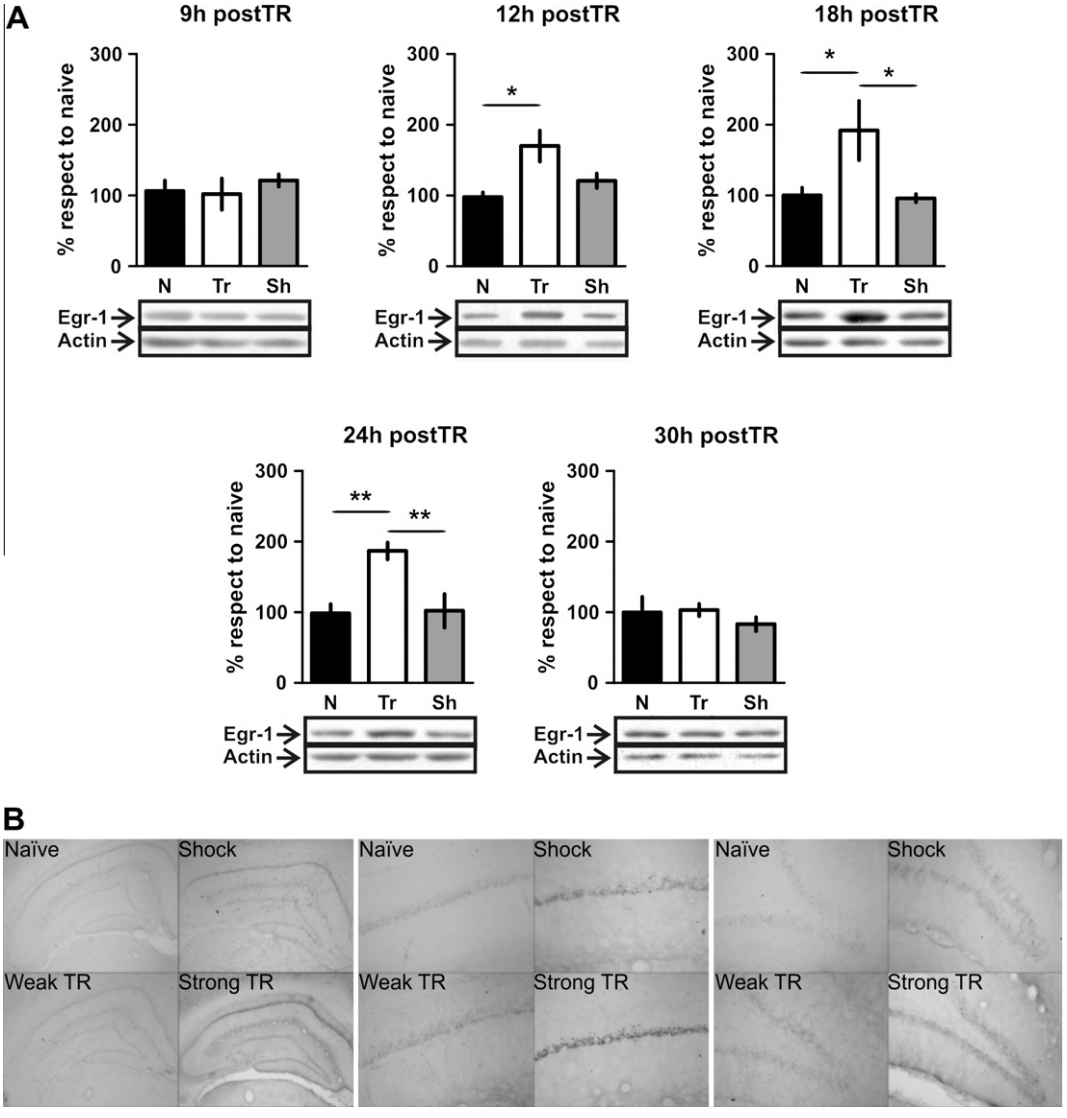

Fig. 1. IA training induces a delayed wave of Egr-1 in the CA1 of dorsal hippocampus. (A) Time course of hippocampal Egr-1 levels late after strong IA training. Bars indicate the percentage of change respect to the naïve (N) and shocked (Sh) groups for rats trained (Tr) and sacrificed 9, 12, 18, 24 or 30 h after the behavioral procedure. Data are expressed as mean ± SEM of Egr-1/Actin ratio. $^{*}p < 0.05$; $^{**}p < 0.01$; Newman–Keuls test after ANOVA, $n = 5–6$ per group. (B) Egr-1 immunoreactivity is increase in CA1 region of dorsal hippocampus after strong IA training. Rats were sacrificed 12 h after IA training, coronal sections of the brain were subjected to immunohistochemical analysis using antibodies against Egr-1. Representative photomicrographs show Egr-1 immunoreactivity in the dorsal hippocampus (Hp, left panel). Inset at a high magnification showing the CA1 region of the dorsal hippocampus (middle panel) and dentate gyrus (DG, right panel).