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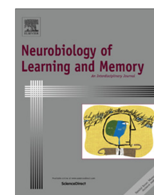

Mapping fear memory consolidation and extinction-specific expression of JunB

Kasia Radwanska [a,b,*], Grace Schenatto-Pereira [b,c], Magdalena Ziółkowska [a], Kacper Łukasiewicz [a], K. Peter Giese [b]

[a] Laboratory of Molecular Basis of Behavior, Nencki Institute, ul. L. Pasteura 3, Warsaw, Poland
[b] Centre for the Cellular Basis of Behavior, MRC Centre for Neurodegeneration Research, Institute of Psychiatry, King's College London, James Black Centre, 125 Coldharbour Lane, London SE5 8AF, UK
[c] Núcleo de Neurociências (NNC), Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, Belo Horizonte, Minas Gerais, Brazil

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ABSTRACT

Understanding the molecular and cellular process specifically regulated during fear memory consolidation and extinction is a critical step toward development of new strategies in the treatment of human fear disorders. Here we used inhibitory component of AP-1 transcription factor, JunB, in order to map brain regions where JunB-dependent transcription is regulated during consolidation and extinction of contextual fear memory. We found that contextual fear memory consolidation induced JunB expression in the medial nucleus and intercalated cells of the amygdala while extinction training induced JunB in the CA1 and CA3 areas of the dorsal hippocampus. JunB upregulation induced by contextual fear memory extinction was absent in alphaCaMKII autophosphorylation-deficient mice which have impaired contextual fear memory extinction. Thus, our data suggest that JunB expression in the medial nucleus and intercalated cells of the amygdala is involved in fear memory consolidation while alphaCaMKII-autophosphorylation-dependent JunB expression in the areas CA1 and CA3 of the dorsal hippocampus regulates fear memory extinction.

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1. Introduction

Both fear of danger and its suppression are evolutionary adaptive. Dysregulation of fear memory formation or extinction results in serious psychiatric conditions such as phobias or post-traumatic stress disorder. Thus, neurobiological mechanisms that mediate long-term storage, retrieval and extinction of fear memory are of considerable interest. One of the outstanding questions of the neurobiology of memory is to what extent fear memory extinction involves or recapitulates molecular and neuronal processes underlying memory formation. Here, in order to approach this question we mapped expression of immediate early transcription factor JunB induced by fear memory consolidation and extinction.

Fear memory consolidation and extinction require gene expression and new protein synthesis (Hernandez & Abel, 2008; Jarome & Helmstetter, 2014; Mizuno & Giese, 2005). Accordingly, multiple transcription factors such as AP-1, CREB or MEF2, were found to regulate neuronal plasticity (Josselyn, 2010; Liu et al., 2008; Mizuno & Giese, 2005; Nestler, 2008; Rashid, Cole, & Josselyn, 2014; Robison & Nestler, 2011) and expression of plasticity-related genes (Kaczmarek, Lapinska-Dzwonek, & Szymczak, 2002; McClung & Nestler, 2003; Rylski et al., 2009). AP-1 acts as a dimer and can be composed of Fos (c-Fos, FosB and ΔFosB) and Jun (JunB, c-Jun and JunD) family proteins. The function of AP-1 complex depends on its composition (Shaulian & Karin, 2001; Szabowski et al., 2000). c-Fos protein, a key component of AP-1 transcription factor, is possibly the most commonly used marker of neuronal activity. A number of papers used mapping of c-Fos expression as a tool to identify brain regions activated during memory consolidation, reconsolidation and extinction (see for review Knapska, Radwanska, Werka, & Kaczmarek, 2007). Importantly, the function of c-Fos-expressing brain regions in memory processes was confirmed with multiple experimental approaches including the most recent experiments applying optogenetic regulations (Liu et al., 2012; Redondo et al., 2014; Shima et al., 2013). At the same time much less is known regarding the function of c-Fos partners from Jun family, and Jun-containing AP-1 complexes in the regulation of learning and memory. Several studies showed however that both drugs of abuse and behavioral training induce expression of c-Jun, JunB and JunD proteins (Guedea et al., 2011; Radwanska, Caboche, & Kaczmarek,

* Corresponding author at: Laboratory of Molecular Basis of Behavior, Nencki Institute, Pasteura 3, 02-093 Warsaw, Poland. Fax: +48 (22) 822 53 42.
E-mail address: kradwans@nencki.gov.pl (K. Radwanska).

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2005; Radwanska, Valjent, Trzaskos, Caboche, & Kaczmarek, 2006; Radwanska et al., 2008; Sherrin et al., 2010; Strekalova et al., 2003). Furthermore, c-Jun-N-terminal kinases were shown to be important regulators of associative learning (Kenney, Florian, Portugal, Abel, & Gould, 2010; Sherrin et al., 2010).

Here, in order to investigate the molecular correlates of context memory consolidation and extinction we employed mapping of the expression of JunB transcription factor in wild-type mice as well as in alphaCaMKII autophosphorylation-deficient mutants, which are impaired in fear memory extinction (Kimura, Silva, & Ohno, 2008; Radwanska et al., 2011). Our findings indicate differential pattern of JunB expression during fear memory formation and extinction. In the former process upregulation of JunB expression is observed in the intercalated cells and medial nucleus of the amygdala while in the later in the hippocampus, supporting the hypothesis of process-specific activation of the brain circuits. Furthermore, we show that extinction-induced JunB expression is compromised in alphaCaMKII autophosphorylation-deficient mutant mice. In conclusion, our data suggest that JunB protein expression in the amygdala is involved in fear memory formation, while alphaCaMKII-regulated JunB expression in the hippocampus appears to have a role in memory extinction.

2. Materials and methods

2.1. Animals

The mice were housed in groups of two to six and maintained on a 12 h light/dark cycle with food and water *ad libitum*. The *αCaMKII-T286A* mice were generated and PCR genotyped as previously described (Giese, Fedorov, Filipkowski, & Silva, 1998). All of the mice for the behavioral studies were 3–5 months old at the time of training. All experiments used approximately equal numbers of male and female mice and were undertaken in accordance with the UK Animals (Scientific Procedures) Act 1986 and the Animal Protection Act of Poland.

2.2. Contextual fear conditioning

The animals were trained in a conditioning chamber (Med Associates Inc, St Albans, USA) in a soundproof box. The chamber floor had a stainless steel grid for shock delivery. Prior to training the chamber was cleaned with 70% ethanol and a paper towel soaked in ethanol was placed under the grid floor. In order to camouflage any noise in the behavioral room background noise was supplied to the chamber by a white noise generator positioned in the side of the soundproof box.

On the conditioning day, the mice were brought from the housing room into a holding room where they were allowed to acclimatize for 30 min before training. Next, mice were placed in the chamber and after a 148 s introductory period a foot shock (2 s, 0.7 mA) was presented. The shock was repeated 5 times, with inter-trial interval 90 s. Thirty seconds after the last shock the mouse was returned to its home cage.

Contextual fear memory was tested and extinguished 24 h after training by re-exposing the mouse to the conditioning chamber for 30 min, followed by three 30-min testing sessions during three consecutive days. A video camera was fixed inside the door of the sounds attenuating box, which allowed the behavior to be observed and scored. Freezing behavior (defined as complete lack of movement, except for respiration) and locomotor activity of mice were automatically scored by computer.

The animals used for JunB immunohistochemistry were anesthetized (i.p. sodium pentobarbital; 50 mg/kg, Sigma–Aldrich) 120 min after the beginning of the training (TRAINED) or first extinction session (EXT). As controls were used naïve animals taken from their home cages, animals sacrificed two hours after exposure to the experimental chamber without shock presentation (CTX) or mice anesthetized twenty-six hours after training (without re-exposure to the experimental context) (5US).

2.3. Immunohistochemistry

Immunohistochemical procedures were based on those previously described by Radwanska et al. (2005). The mice were anaesthetized and perfused with PBS (Sigma–Aldrich) followed by 4% PFA (Sigma–Aldrich) in PBS. The brains were then removed and placed overnight in the same fixing solution and afterward in 30% sucrose in PBS for three days. Coronal brain cryo-sections (40 μm thick) were prepared (Microm HM560) and stored at −20 °C in PBSAF [PBS, 20% sucrose (Sigma–Aldrich), 15% ethylene glycol (Sigma–Aldrich), 0.05% NaN_3 (Sigma–Aldrich)]. The sections were washed three times in PBS before being placed in hydrogen peroxide (3% H_2O_2/PBS) for six minutes. Next, the sections were washed three times for six minutes in PBS/0.3% Triton X-100 (Sigma–Aldrich) followed by 2 h of incubation in a blocking solution (3% NGS in PBS/0.3% Triton X-100). After 3 washes for 6 min in PBS with 0.3% Triton, the sections were incubated with the selective antibodies directed against JunB (sc-46, Santa Cruz Biotechnology; 1:200, overnight at 4 °C). After the incubation with the primary antibody, the sections were washed 3 times for 6 min in PBS with 0.3% Triton X-100 and incubated with the secondary antibody (1:1000, biotynylated anti-IgG antibody; Vector Laboratories) for one hour at room temperature. After incubation with the secondary antibody, the sections were washed 3 times for 6 min in PBS with 0.3% Triton X-100 and incubated with avidin–biotin horseradish peroxidase complex (1:1000 in TBS; Vector Laboratories). The sections were washed 3 times for 6 min in PBS and finally developed with 1 mg/ml diaminobenzidine/0.005% H_2O_2 in PBS for 30 min. Next, the sections were washed 3 times in PBS to stop the reaction. Finally, the sections were mounted on poly-lysine-covered slides (Sigma–Aldrich), air-dried, dehydrated in ethanol solutions and xylene, and embedded in Entellan (Merck).

DAB Immunostaining was analyzed with AxioImager Z1 microscope, with Plan-Apochromat x2.5 objective. Photomicrograph of stained brain sections were taken with a digital camera (AxioCam MRm, Zeiss). The TIFF-format micrographs were analyzed using ImageJ (NIH). The threshold tool was used which identifies objects distinct to the background in an image based on coloring and intensity. For each image, the threshold was adjusted, an area of interested was selected, and the percentage-area of a selection highlighted by threshold was measured. Every 6th section from the dorsal hippocampus, prefrontal cortex and amygdala was analyzed. The position of the analyzed brain regions was determined according to the atlas of the mouse brain (Paxinos & Franklin, 2001).

2.4. Statistics

All data were analyzed using GraphPad Prism software. For statistical analysis two-way analysis of variance (ANOVA), and Tukey's post hoc or Sidak's multiple comparisons post hoc tests were used when appropriate.

3. Results

3.1. CaMKII autophosphorylation regulates fear memory extinction

Previously, we have shown that alphaCaMKII autophosphorylation deficient mice (T286A) have impaired fear memory extinction

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