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BRAIN RESEARCH

Research Report

Temporal gene expression profile of the hippocampus following trace fear conditioning

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ABSTRACT

In this paper we report the results of gene expression profiling of C57Bl/6N mice hippocampus after trace fear conditioning (TFC), and the identification of genes regulated at early and late steps after conditioning. Several of the genes regulated at early steps following TFC appeared common to many training protocols. At later stages (2 and 6 h), most of the genes identified were different from those identified following other learning paradigms resulting in memory consolidation. At 6 h after training, few genes were upregulated in respect to the naïve condition, suggesting that many gene products have eventually to be downregulated to achieve stable synapses modification and memory formation. In conclusion, the results presented highlight a number of genes whose expression is specifically modified in the mouse hippocampus following TFC and demonstrate the specificity associated to different forms of conditioning.

1. Introduction

Memory formation involves translational, transcriptional and epigenetic changes driven by the postsynaptic activation of neurotransmitter receptors. From mollusks to mammals, memory can be schematically divided into a short-term (STM), and long-term memory (LTM). STM, which lasts from minutes to few hours, is protein and RNA synthesis independent and involves posttranscriptional modification of existing molecules. The formation of LTM requires several hours and new protein and RNA synthesis that sequentially occur at precise times during the process (Abel and Lattal, 2001). Several studies have applied the cDNA microarray technology to define gene expression profiles induced by behavioral training in animal models, in wild type and in mutant animals (Cavallaro et al., 2002; Donahue et al., 2002; Levenson et al., 2004; Robles et al., 2003). There was in general little agreement among the lists of genes identified after different training conditions. Possibly the differences were due to the animal strain, to the time points and the tissues or brain regions

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Abbreviations: TFC, trace fear conditioning; STM, short-term memory; LTM, long-term memory; CS, conditioned stimulus; US, unconditioned stimulus; CTX, context; GO, gene ontology; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase

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examined. Alternatively, and more likely, the different tests could involve different gene pathways and accurate experimental design would be required for comparative analysis.

The present study focused on gene regulation in the hippocampus following TFC (Huerta et al., 2000), a conditioning paradigm where a neutral stimulus (conditioned stimulus, CS, usually a tone) is paired with an aversive event (unconditioned stimulus, US, generally a foot shock) separated by a short time interval. In all cases, fear conditioning is induced mainly through the involvement of the amygdala function (Fanselow and Poulos, 2005; Maren and Quirk, 2004), but also the hippocampus is involved as context conditioning, which is always associated with US conditioning, is mainly hippocampus dependent (Fanselow, 2000). In addition, TFC was shown to depend on intact hippocampus and prefrontal cortex functions (McEchron et al., 1998; Runyan et al., 2004) to maintain the association between the CS and US through the trace period. Several studies reported gene expression profile of hippocampus or amygdala following the classical form of fear conditioning. Gene expression was studied after the training session (Keeley et al., 2006), after contextual and cued fear memory retention (Mei et al., 2005) or after US fear conditioning training (Levenson et al., 2004). No information is available for TFC.

We report here the results of the study of C57Bl/6N mice hippocampus at different time points after conditioning and we show that some of the genes previously found to be regulated after classical fear conditioning were also changed in TFC, suggesting common molecular pathways. However many differences were also clearly detected, in agreement with the different roles of the hippocampus in the different versions of the test.

2. Results

2.1. *Trace fear conditioning*

TFC is schematically represented in Fig. 1A (see Experimental procedures for details). The percentage of freezing over the five CS tones presentation increased in mice during the conditioning session (CS effect: $F[4, 212]=87.9$, $p<0.0001$) (Fig. 1B), as a result of associative fear-related memory formation. Under those conditions, a robust LTM was formed if mice were re-exposed to CS in a different context (Wanisch et al., 2005).

2.2. *Microarray analysis*

To follow gene expression in the hippocampus during LTM formation, we analyzed total RNA extracted at 0.5, 2 and 6 h after the end of the TFC training and we compared the expression profiles with that of naïve littermates that remained in their home cages throughout the experiment (Fig. 1A).

We used 18 mice for each experimental condition. As three trace fear conditioning experiments were independently replicated to obtain the final number of animals to test, to account for variability among animals and among experiments, identical aliquots of RNA from purified hippocampus of single animals were pooled in 3 pools of 6 animals. Each pool contained animals trained in each of the training sessions. The RNA pools were hybridized to the Affymetrix Mouse 430A 2.0 array containing 22,690 probe sets, corresponding to approximately 14,000 well-characterized genes. Probeset levels were generated using the GCRMA method (Irizarry et al., 2003) and normalized using quantile normalization (Bolstad et al., 2003) at the probe level. To eliminate background data, a filter was applied based on detection calls as described in Experimental procedures.

The Limma Bioconductor library (Smyth, 2004) was used for the selection of differentially expressed genes among the 13 054 probe sets that remained after the filtering procedure. By comparison with the naïve mice, we identified 79 differentially expressed genes ($p<0.05$): 10 genes were upregulated after 0.5 h, 20 genes were modulated after 2 h and 55 genes after 6 h (Table 1 and Table S1). Few genes maintained their modified status for more than one time point. *c-fos* and *Egr1* were upregulated at 0.5 h and remained upregulated at 2 h whereas *Tsc22d3*, *Hspb1* and *Hexim1* were upregulated at 2 h and 6 h. The most striking difference between the three time points can be however found in the temporal pattern of gene regulation (Fig. 2). At 0.5 h, all differentially expressed genes were upregulated, after 2 h most of the genes (16 out of 20) were still upregulated, whereas at 6 h the number of down-regulated genes was dramatically increased to 76% (42 out 55) of all the differentially expressed genes.

Early genes were predominately transcription factors. Among the genes differentially expressed at 2 h many were involved in the process of protein folding and protein quality

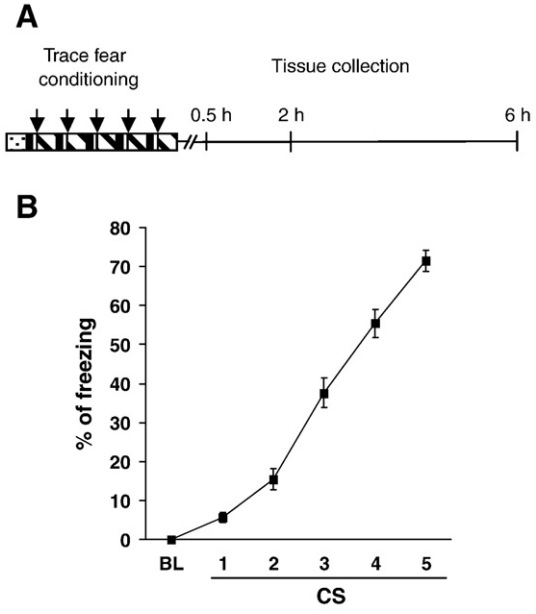

Fig. 1 – Schematic representation of the trace fear conditioning training protocol. (A) Dotted box: 60 s habituation; black box: 15 s CS; white box: 15 s trace; arrow: 2 s US and striped box: 60 s ITI. After the training, animals were returned to their home cage, before hippocampus dissection at the indicated time points. (B) Percentage of freezing during the CS presentation. Data points represent the freezing mean and error bars indicate SEM ($n=54$).