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# Hippocampal gene expression profiles in passive avoidance conditioning

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## Abstract

Many experiments in the past have demonstrated the requirement of *de novo* gene expression during the long-term retention of learning and memory. Although previous studies implicated individual genes or genetic pathways in learning and memory, the collective behaviours of the genes is mostly unknown. We have used genome-scale screening by microarray analysis to examine the hippocampal expression of more than 1200 genes relevant to neurobiology during instrumental conditioning. Training rats on a step-through passive avoidance task led to unique patterns of gene expression when compared to naïve animals or those exposed to the conditioned or the unconditioned stimulus alone. The newly identified genes afford a quantitative view of the changes which accompany conditioning at the genomic level and enable deeper insights into the molecular basis underlying learning and memory.

## Introduction

Two general forms of memory have been classified by their duration: short-term memory (STM), which is rapidly formed and can outlast training for minutes or hours, and long-term memory (LTM), which lasts from hours to days, weeks or even years. STM involves post-translational modifications of preexisting molecules, which alters the efficiency of synaptic transmission. In contrast, LTM can be blocked by inhibitors of transcription or translation indicating that it is dependent on *de novo* gene expression (Davis & Squire, 1984; Stork & Welzl, 1999). Proteins newly synthesized during memory consolidation may contribute to restructuring processes at the synapse and thereby alter the efficiency of synaptic transmission beyond the duration of short-term memory. Revealing the dependence of LTM on protein synthesis, however, provides no information about the identity and specificity of the required proteins. Because the quantity of a particular protein is often reflected by the abundance of its mRNA, a variety of methods has been used to describe a limited number of differentially expressed mRNAs during LTM. Increased or, less often, decreased expression of genes has been demonstrated during specific time windows following learning (Stork & Welzl, 1999).

In the past we have used RNA fingerprinting to identify genes which were up-regulated in the hippocampus of water maze-trained rats (Cavallaro *et al*., 1997). Spatial learning-induced changes in expression of some of these genes occur at selective times and in specific hippocampal subfields (Cavallaro *et al*., 1997; Zhao *et al*., 2000), indicating distinct contributions to learning and memory. Increased expression of one of these genes, the ryanodine receptor type-2, could result in increased mobilization of $[Ca^{2+}]$ which may participate in the synaptic changes underlying associative memory storage (Alkon *et al*., 1998). In these past studies, however, we screened only a small fraction of the genes which may have been differentially expressed during long-term memory. Thus, the questions remain how many and which genes are involved in memory and how do they interact functionally to effect memory storage. In addition, each of the identified genes may not act in a linear sequence but in complex networks. Successive screening therefore may be needed to uncover the networks of genes involved in distinct steps of memory storage.

To begin a comprehensive survey of the gene-based molecular mechanisms which underlie long-term memory, we have recently used the unprecedented experimental opportunities which genome sequences and the development of cDNA microarray technology now provide (Cavallaro *et al*., 2002a; D'Agata & Cavallaro, 2002) to perform genome-wide expression analysis in classical conditioning of the rabbit nictitating membrane response (Cavallaro *et al*., 2001) and water maze learning (Cavallaro *et al*., 2002b). In the present study we have extended these experiments to analyse the patterns of gene regulation during passive avoidance learning.

## Materials and methods

### *Subjects*

The subjects were 32 adult male Wistar rats each weighing 200–300 g. Rats were given access to food and water *ad libitum*, and maintained on a 12-h light–dark cycle in a constant temperature (23 °C). Behavioural tests were carried out in the light phase, were approved in advance by the Animal Care and Use Committee and are consistent with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### *Passive avoidance test*

Passive avoidance tests was performed as previously described (Shirayama *et al*., 2002) by using a passive avoidance apparatus (San Diego Instruments, Inc., San Diego, CA, USA). The apparatus was divided into two compartments by a retractable door: a lit safe compartment and a darkened shock compartment. The first group of animals (naïve, $n = 8$) were maintained in their cage and were not trained. The rest of the animals experienced a 2-day behavioural training. The first day, the animals were handled for 2 min and then

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placed into the safe compartment and allowed to explore both chambers of the apparatus for 3 min. The second day, in the training trial, the second group of animals [termed 'conditioned animals' (CA); $n = 8$] were placed in the safe compartment with the door closed. After 2 min of acclimatization the light was turned on, the door opened and the animal was allowed to enter the dark compartment. After the rat stepped completely with all four paws into the dark compartment, the door was closed, and a mild inescapable foot shock (0.5 mA, 2 s duration) was delivered from the grid floor. Following the shock, the rat was removed and returned to its home cage. The second day, the third group of animals [termed 'conditioned stimulus-trained animals' (CSTA); $n = 8$] were placed into the safe compartment. After 2 min of acclimatization the light was turned on, the door opened and the animal allowed to enter the dark compartment. After the rat stepped into the dark compartment, the door was closed but no foot shock was delivered from the grid floor. Then the rats returned to their home cages. The fourth group of animals [termed 'unconditioned stimulus-trained animals' (USTA); $n = 8$] were placed in one of the two dark compartments. They were allowed to move freely to both compartments. After 2 min of acclimatization they received an escapable foot shock (0.5 mA, 2 s duration) and then returned to their home cages. Six hours later, half of the animals ($n = 4$ for each of the four experimental groups) were killed with an overdose of pentobarbital (30 mg/kg) followed by decapitation, and their hippocampi were rapidly dissected and frozen on dry ice.

Twenty-four hours after the training trial, the other half of the animals ($n = 4$ for each of the four experimental groups) performed the retention test. The animals were placed in the safe compartment with the door closed. After 2 min of acclimatization the light was turned on, the door opened and the animal was allowed to enter the dark compartment. The latency to enter the dark compartment was recorded and used as the measure of retention. The rats avoiding the dark compartment for $>300$ s were considered to have a memory of the training experience. Stimulus delivery and data collection were via a Pentium PC computer equipped with the Gemini Avoidance System software (San Diego Instruments, Inc., San Diego, CA, USA).

### *Microarray analysis*

Total hippocampal RNA from each animal ($n = 4$ for each of the four experimental groups) was extracted, reverse-transcribed, biotinylated and hybridized to a single Affymetrix GeneChip Rat Neurobiology U34 array as previously described (Cavallaro *et al*., 2002b) with the protocol outlined in the GeneChip Expression Analysis technical manual (Affymetrix, CA, USA). The arrays were washed and stained by using a fluidics system with streptavidin–phycoerythrin (Molecular Probes, Eugene, OR, USA), amplified with biotinylated antistreptavidin antibody (Vector Laboratories, Burlingame, CA, USA) and then scanned with a GeneArray Scanner (Affymetrix). To determine the quality of labelled targets prior to analysis on GeneChip Rat Neurobiology U34 arrays, each sample was hybridized to one GeneChip Test3 array. The image data were analysed by MicroArray Suite 4.0 Gene Expression analysis program (Affymetrix). Normalization, filtering, statistical and cluster analysis of the data were performed with the GeneSpring 4.2 software (Silicon Genetics, CA, USA). Detailed protocols for data analysis of Affymetrix microarrays and extensive documentation of the sensitivity and quantitative aspects of the method have been described (Lipshutz *et al*., 1999). The average correlation coefficient between raw data of individual replicates ($n = 4$) in the same experimental condition were as follows: naïve, 0.99; CSTA, 0.99; USTA, 0.995; CA, 0.973. The raw data from each array was normalized in the following way: each measurement for each gene was divided by the 50th percentile of all measurements. Each gene was then normalized to itself by making a synthetic positive control for that gene, and dividing all measurements for that gene by this positive control. This synthetic control was the median of the gene's expression values over all the samples. Average difference values of $>0$ represent probe sets where the intensity of the mismatched probe was on average greater than the perfect matched probe and thus the probe set was performing poorly. For this reason, normalized values below 0 were set to 0. To test for statistically significant changes in signal intensity, genes with an average change $>2$ fold were screened by Welch's ANOVA ($P < 0.05$) using the Benjamini and Hochberg False Discovery Rate procedure to adjust for multiple comparisons. Pairwise comparisons were performed with Dunnett's test.

### *Real-time quantitative RT-PCR*

To further confirm the reliability of the array data, the mRNA levels of 16 genes were quantified by real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR). Five of these genes were randomly selected among those genes differentially expressed between naïve and CA; the rest (11) were not differentially expressed in conditioned animals and were randomly selected among those genes previously implicated in water maze learning (Cavallaro *et al*., 2002b). Aliquots of cDNA (0.1 and 0.2 μg) from individual animals (four animals/group), and known amounts of external standards (purified PCR product, $10^2$–$10^8$ copies) were amplified in parallel reactions using primers shown in Table 1. PCR amplifications were performed as previously described (Cavallaro *et al*., 2002b). Specificity of PCR products obtained was characterized by melting-curve analysis followed by gel electrophoresis and DNA sequencing.

## Results

To relate mRNA induction to a learning task we trained the rats using a behavioural protocol, a step-through passive avoidance test, known to require hippocampus-dependent learning and depend upon transcription (Stubley-Weatherly *et al*., 1996). In these experiments, conditioned animals (CA) were trained to avoid moving from the lighted to the darkened section of a conditioning chamber by delivering a foot shock when they entered the darkened section. Control rats included untrained (naïve) animals, and animals exposed to the unconditioned (USTA) or the conditioned (CSTA) stimulus. To verify that the trained rats in fact learned the passive avoidance task, learning was assessed in a comparable group of animals by evaluating the latency of step-through in a retention test. Twenty-four hours after the one-trial training period, only CA learned to associate stepping through the darkened chamber with the foot shock (Fig. 1).

Hippocampal gene expression profiles in CA, USTA, CSTA and naïve animals were measured 6 h after training using microarrays containing 1263 genes relevant to neurobiology. The complete set of data is available available online (http://web.tiscali.it/sebastiano_cavallaro). Gene expression data in each of the four experimental conditions represented the average from four separate microarray analyses performed on hippocampal RNA samples from individual animals. To confirm the reliability of the array data we selected 16 genes and quantitatively validated their differential expression in hippocampal mRNA of individual animals using real-time quantitative RT-PCR (Table 1). Remarkably, the pattern of gene expression from sample to sample observed by microarrays closely paralleled the pattern observed using real-time RT-PCR. The mean ± SD of the correlation coefficients between the two profiles was $0.94 \pm 0.07$ (Table 1).

When gene expression profiles of naïve animals were compared to those of CSTA or USTA, 46 and 60 genes, respectively, were found

TABLE 1. Validation of microarray data in individual animals by real-time quantitative RT-PCR

| Gene | | RNA expression levels in different experimental conditions | | | | Correlation coefficient | Quantitative RT-PCR | | Length (bp) |
|---|---|---|---|---|---|---|---|---|---|
| Name | GenBank | Naive | CSTA | USTA | CA | | Forward primer | Reverse primer | |
| Caspase 6 | AF025670 | 1.12 ± 0.16<br>2770 ± 233 | 1.01 ± 0.14<br>2750 ± 291 | 1.30 ± 0.29<br>3170 ± 322 | 1.00 ± 0.25<br>2506 ± 310 | 0.94 | CCGAGCAGTACAAGATGGAC | CTCTGAGAACCTTCGAGTCG | 140 |
| Frequenin homolog | L27421 | 0.93 ± 0.07<br>6081 ± 574 | 1.01 ± 0.04<br>6788 ± 631 | 1.07 ± 0.08<br>6821 ± 502 | 0.80 ± 0.14<br>5890 ± 663 | 0.94 | CTGAAGTTGTGGAGGAGCTG | CTTGTTCTCGTCGAAGACG | 200 |
| Glutamate receptor, NMDA 1 subunit | S39221 | 0.98 ± 0.15<br>2007 ± 148 | 1.31 ± 0.04<br>2579 ± 344 | 1.32 ± 0.09<br>2411 ± 320 | 0.87 ± 0.10<br>1772 ± 215 | 0.98 | CAGAGGCGGTGAACATTCTA | TGAGTGAACATGGCTCCTTC | 139 |
| GnRH receptor | U92469 | 0.85 ± 0.34<br>3412 ± 317 | 1.34 ± 0.35<br>5069 ± 478 | 0.76 ± 0.26<br>3283 ± 291 | 0.94 ± 0.37<br>3637 ± 218 | 0.99 | ACTGCTCAGCCATCAACAAC | AGAGTCTCAAGGAGGTTGGC | 238 |
| Kcnj11 potassium inwardly-rectifying channel | D86039 | 0.84 ± 0.08<br>4782 ± 518 | 1.00 ± 0.00<br>4914 ± 559 | 1.00 ± 0.05<br>4855 ± 363 | 1.09 ± 0.07<br>5002 ± 624 | 0.93 | CAGCCACGACAGGATAAGTT | GGTAGAGGACAGAGACACGC | 132 |
| Insulin-like growth factor II | X16703 | 0.84 ± 0.43<br>1088 ± 242 | 2.53 ± 1.32<br>3201 ± 109 | 1.73 ± 1.70<br>2317 ± 253 | 1.92 ± 1.11<br>2776 ± 265 | 0.99 | TTCGACACCTGGAGACAGTC | CTGCTCAAGAGGAGGTCACA | 259 |
| Interleukin 12b | S82489 | 0.85 ± 0.17<br>420 ± 36 | 0.72 ± 0.05<br>395 ± 14 | 1.37 ± 0.27<br>726 ± 59 | 1.81 ± 0.49<br>1602 ± 94 | 0.94 | TGTTGTAGAGGTGGACTGGC | GTGGAGCAGCAGATGTGAGT | 223 |
| Leptin receptor | AA998983 | 1.27 ± 0.17<br>2622 ± 371 | 0.53 ± 0.32<br>1628 ± 180 | 0.97 ± 0.92<br>2411 ± 275 | 0.80 ± 0.28<br>2085 ± 199 | 0.98 | TTAACAGTGTTCCGAGCAGC | GGCTGTATGTCATTGTACCG | 63 |
| Neurotrophin 5 (neurotrophin 4/5) | M86742 | 2.34 ± 0.92<br>406 ± 41 | 1.16 ± 0.18<br>263 ± 15 | 0.92 ± 0.09<br>211 ± 11 | 0.81 ± 0.13<br>149 ± 17 | 0.97 | GAGGCACTGGCTCTCAGAAT | GTAGAGCAGTCGAACCATCC | 233 |
| Neuropeptide Y5 receptor | U66274 | 0.62 ± 0.22<br>269 ± 28 | 1.17 ± 0.40<br>501 ± 35 | 1.12 ± 0.05<br>477 ± 44 | 1.00 ± 0.09<br>366 ± 27 | 0.96 | AGGACTACAGAGGCAGCGTA | CCAACAAGACAGAGGTCAG | 222 |
| p38 mitogen activated protein kinase | U73142 | 0.37 ± 0.24<br>1609 ± 122 | 1.38 ± 0.66<br>5538 ± 571 | 1.28 ± 0.06<br>5603 ± 688 | 1.04 ± 0.14<br>3021 ± 221 | 0.93 | GCAGACCTGAACAACATCGT | GGATTATGTCAGCCGAGTGT | 112 |
| Phospholipase A2, group VI | U51898 | 0.95 ± 0.07<br>1925 ± 122 | 0.86 ± 0.01<br>1809 ± 164 | 0.98 ± 0.24<br>2063 ± 175 | 1.04 ± 0.17<br>2188 ± 174 | 0.97 | CCTCAAGTGCCTGTAACCTG | GCATGATGTCTGAGCCTAGC | 214 |
| Potassium inwardly-rectifying channel,subfamily J, member 2 | AF021137 | 0.86 ± 0.26<br>1996 ± 225 | 1.17 ± 0.31<br>2207 ± 186 | 1.31 ± 1.16<br>2576 ± 243 | 1.17 ± 0.44<br>2103 ± 266 | 0.83 | GTGAGAACCAACCGCTACAG | CGGATGTCCACACAGGTAG | 230 |
| Transforming growth factor, beta receptor III | M80784 | 1.47 ± 0.39<br>2737 ± 368 | 1.60 ± 0.36<br>2929 ± 317 | 1.85 ± 1.23<br>3070 ± 424 | 0.60 ± 0.12<br>1259 ± 73 | 0.99 | GTTGGAGAGATGGCAGTGAC | GCGTTGATTGGTGACAGTTC | 122 |
| Tumor necrosis factor (TNF-alpha) | L00981 | 1.15 ± 0.27<br>156 ± 13 | 0.94 ± 0.38<br>136 ± 12 | 0.97 ± 0.01<br>145 ± 10 | 1.19 ± 0.13<br>170 ± 21 | 0.94 | CAGAGGAGCTTGTGAGCAGT | CTGAGGAGTTCCAGTGAACG | 139 |
| Vimentin | X62952 | 1.58 ± 0.51<br>830 ± 66 | 0.92 ± 0.07<br>692 ± 53 | 1.41 ± 0.40<br>711 ± 64 | 0.86 ± 0.12<br>724 ± 33 | 0.71 | CTTCTCTGGCACGTCTTGAC | TCCTGGATCTCTTCATCGTG | 94 |

RNA expression levels: each gene has two sets of values quoted, the upper ones being the microarray average normalized value ± SD and the lower being quantitative RT-PCR average ± SD of copies/100pg RT-RNA. RNA levels for the indicated genes were assayed by real-time quantitative RT-PCR as previously described (Cavallaro *et al.*, 2002b). The sequence of the primers used and the length of PCR products generated are indicated. Results are absolute RNA levels (copy number) from animals in four experimental condition: untrained rats (naïve), animals exposed to the conditioned (CSTA) or the unconditioned (USTA) stimulus, and conditioned animals (CA).

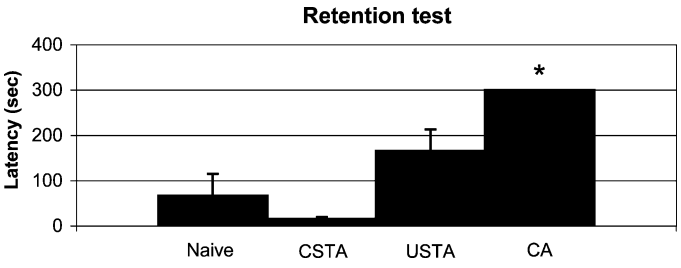


FIG. 1. Passive avoidance retention test. Conditioned animals (CA) were trained to avoid moving from the lighted to the darkened section of a conditioning chamber by the delivery of a foot shock when they entered the darkened section. Control rats included untrained (naïve) animals, and animals exposed to the conditioned (CSTA) or the unconditioned (USTA) stimulus. Twenty-four hours after the training trial, half of the animals ($n = 4$ per group) performed the retention test to verify that the trained rats in fact learned the passive avoidance task. The animals were placed in the safe compartment with the door closed. After 2 min of acclimatization the light was turned on, the door opened and the animal was allowed to enter the dark compartment. The latency to enter the dark compartment was recorded and used as the measure of retention. The rats avoiding the dark compartment for >300 s were considered to have a memory of the training experience. During the retention trial, CA had a longer mean step-through latency than naïve, CSTA and USTA ($^*P < 0.001$).

differentially expressed (Fig. 2A). These genes indicate that physical activity and mild stress associated with behavioural training has a significant impact on hippocampal gene expression.

When gene expression levels in naïve animals were compared to CA, 38 genes (3%) were found differentially expressed and operationally defined as 'memory-related genes' (MRGs) (Fig. 2A). Among MRGs, 21 genes were down-regulated and 17 genes were up-regulated. Some of these MRGs (21/38) were also differentially expressed in CSTA (16) and USTA (16) (Fig. 2A and B).

A hierarchical clustering method was used to group MRGs on the basis of similarity in their expression patterns (Fig. 2B). Genes represented by more than one probe set on the array, such as phosphoinositide 3-kinase p85 and voltage-gated $Na^+$ channel type IV alpha were clustered next to or in the immediate vicinity of each other, indicating that the effects of experimental noise or artifact were negligible (Fig. 2B).

The most evident traits of the clustered data (Fig. 2B) was that MRGs showed entirely different patterns of expression in CA vs. CSTA or USTA. Genes segregating into nine major branches of the dendrogram were assigned to nine clusters (Fig. 2B). Clusters 1–4 represent those genes which were down-regulated, whereas clusters 5–9 include those which were up-regulated in CA. Most MRGs were also differentially expressed in CSTA or USTA (clusters 1, 3–7 and 9), some were differentially expressed only after conditioning (cluster 2 and 8).

Although no information on the identity of the samples was used in the clustering, in some cases genes segregated according to their common biological functions. For example, genes encoding for complement component 3 and 4, serotonin receptor type 1B and 4, and enzymes regulating phosphorylation, such as phosphoinositide 3-kinase p85, p38 mitogen-activated protein kinase and protein phosphatase 1, were expressed concordantly (Fig. 2B).

## Discussion

Our results show that conditioning in the passive avoidance task have profound effects on hippocampal gene expression. Some of the MRGs, those differentially expressed between naïve and CA, were affected by exposing the rats to the conditioned or the unconditioned stimulus alone, whereas others were uniquely induced when the two were associated and the animals were conditioned. Expression changes of MRGs in CSTA or USTA had different magnitudes or more often opposite trends than CA (Fig. 2B, clusters 1–3, 5, 8 and 9). Although learning, physical activity and mild stress associated with behavioural training involve common groups of genes, their behaviour in learning and memory can be distinguished from their cumulative pattern of gene expression as shown in the clustered data.

All of the MRGs identified have a recognized function and can be classified into four major groups based on their translated product (Fig. 2B): (i) cell signalling; (ii) synaptic and cytoskeletal proteins; (iii) apoptosis; and (iv) transcription regulation. Some of these genes have been previously related to synaptic plasticity, memory, or cognitive disorders, whereas others provide a significant number of unique and novel entry points. The exact role and functional relationships of the genes and proteins implicated are presumably not yet recognized. As more time points, behavioural paradigms and pathophysiological conditions are used for similar studies and more complete high-density arrays become available, a more complete interpretive framework will emerge as to the key genes and pathways underlying learning and memory. To facilitate this exploration, the data generated in the present study and those produced in different behavioural (Cavallaro *et al*., 2001; Cavallaro *et al*., 2002b) and pathophysiological (D'Agata *et al*., 2002) conditions are available online (http://web.tiscali.it/sebastiano_cavallaro). Among these conditions, an interesting convergence is evident between two different associative memory protocols, the Morris water maze and the passive avoidance test. Six out 38 MRGs found in the hippocampus of rats after passive avoidance training (Fig. 2B, shown in bold) were also differentially expressed following water maze learning (Cavallaro *et al*., 2002b), suggesting common mechanisms of memory storage in different behavioural paradigms.

In the following paragraphs we will discuss only some of the MRGs implicated by microarray analysis emphasizing those whose regulation in CA was different than CSTA or USTA, and those we previously implicated in water maze learning (Cavallaro *et al*., 2002b).

### *Cell signalling*

The group of genes involved in cell signalling is the largest and includes a subgroup of neuropeptides, growth factors and their receptors. Among these is transforming growth factor (TGF) beta receptor III whose selective down-regulation in CA is in line with previous observation demonstrating impaired learning following administration of TGF beta (Nakazato *et al*., 2002). Differential expression of interleukin 8 receptor beta and interleukin 12b suggest a physiological role of brain cytokines in memory consolidation processes. Recent evidence indicates that interleukins and their receptors are present in the CNS, where they can be involved in a variety of effects including neuroinflammatory processes, modulation of synaptic transmission and regulation of the synaptic connections in the brain (Asensio & Campbell, 1999; Hesselgesser & Horuk, 1999). Although the effects of interleukin-8 and 12 on learning and memory are unknown, other chemokines are known to modulate cognition or are differentially expressed during memory consolidation (Gibertini *et al*., 1995; Ma & Zhu, 1997; Cavallaro *et al*., 2002b; Lynch, 2002).

Among the subgroup of G-protein-coupled receptors and their effectors are three serotonin (5-HT) receptors, the adrenergic alpha 1a and dopamine-1A receptors. Following training, we observed the coordinated reduction of 5-HT1B and 5-HT4, and the selective increase of 5-HT3A receptor mRNA in CA. These data are in agreement with the involvement of the serotonin system through the interplay of different receptor subtypes in learning and memory processes

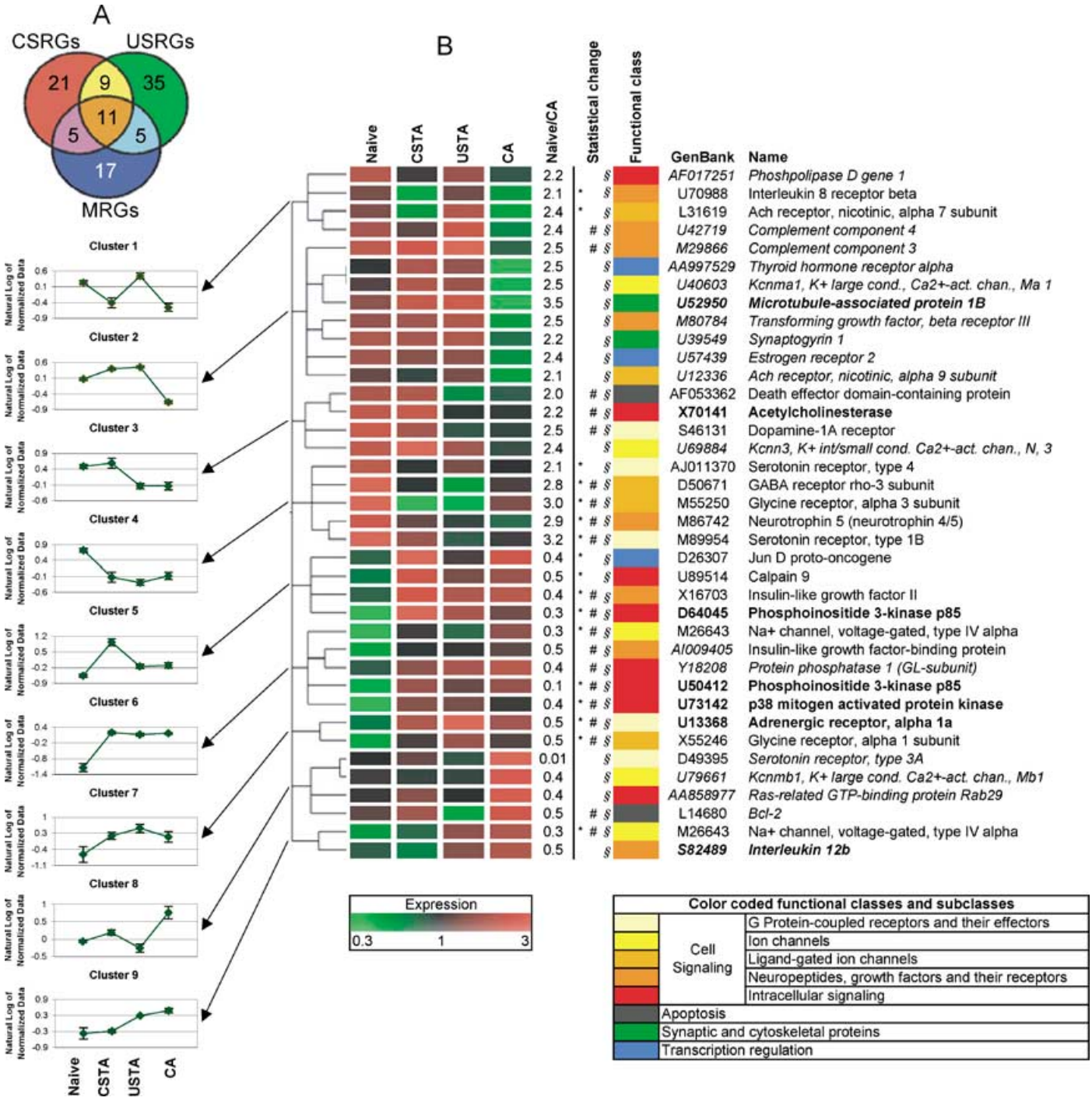


FIG. 2. Differential gene expression during passive avoidance learning. (A) Genes differentially expressed between naïve and CSTA were defined as 'conditioned stimulus-related genes' (CSRGs); genes differentially expressed between naïve and USTA were defined as 'unconditioned stimulus-related genes' (USRGs); genes differentially expressed between naïve and CA were defined as 'memory-related genes' (MRGs). (B) Hierarchical clustering of memory-related genes. A hierarchical clustering algorithm (Pearson correlation, separation ratio 0.2, minimum distance 0.001) was used to order MRGs in a dendrogram in which the pattern and length of the branches reflects the relatedness of the samples. Data are presented in a matrix format: each row represents a single gene and each column an experimental condition. The averaged normalized intensity from four replicates is represented by the colour of the corresponding cell in the matrix. Green, black and red cells, respectively, represent transcript levels below, equal to or above the median abundance across all conditions. Color intensity reflects the magnitude of the deviation from the median (see scale at the bottom). The graphs on the left of the dendrogram represent the averaged natural log of normalized data ± SEM of the genes in nine major clusters. Gene expression ratio between naïve and CA together with statistical significant changes ($^{*}P < 0.05$, naïve vs. CSTA, $^{\#}P < 0.05$, naïve vs. USTA, $^{\S}P < 0.05$, naïve vs. CA) are shown on the right of the matrix. Functional classification of MRGs is represented in a column on the right of the figure where each functional class or subclass is colour-coded. The name and GenBank accession number of MRGs uniquely regulated in CA are indicated in italics, whereas MRGs previously found to be differentially expressed in the hippocampus of water-maze-trained rats (Cavallaro *et al*., 2002b) are indicated in bold.

(Meneses, 1999), in addition to a variety of other behaviours such as emotional states and impulse control (Bouwknecht *et al*., 2001). Administration of 5-HT1B receptor antagonists, for example, is known to prevent memory impairment and facilitate learning, whereas agonists for 5-HT1B generally have opposite effects (Meneses, 1999). Furthermore, 5-HT1B receptor knockout mice exhibit enhanced memory performance (Malleret *et al*., 1999).

The subgroup of ligand-gated ion channels includes the alpha1 and 3 subunits of the strychnine-sensitive glycine-gated chloride channels and the GABAC receptor rho-3 subunit. Differential expression of these ion channels was not selective of CA. They have been involved in a variety of other behaviours (Jentsch *et al*., 2002) and mediate fast postsynaptic inhibition in the brain, and altered expression of their subunits has been shown to modulate hippocampal excitability (Bormann, 2000; Chattipakorn & McMahon, 2002).

The subgroup of ion channels includes three $Ca^{2+}$-activated potassium channels (Kcnma1, Kcnmb1, Kcnn3) and one voltage-gated sodium channel type IV alpha. Differential expression of the three potassium channels, in particular, was significant only in CA. Although the exact contribution of these subunits during learning and memory is unknown, regulation of their expression can produce a flexible tuning of electrical excitability of hippocampal neurons in response to neurotransmitters. Indeed, down-regulation of potassium channels (Kcnma1, Kcnn3) and up-regulation of the sodium channel after passive avoidance training may produce increased excitability.

The subgroup of intracellular signalling proteins includes two kinases, phosphoinositide 3-kinase p85 and p38 mitogen-activated protein kinase, whose expression was significantly increased not only in CA but also in CSTA and USTA. These two signalling enzymes are implicated in a variety of receptor-stimulated cell responses and have been involved in neuronal synaptic plasticity and memory formation (Blum *et al*., 1999; Ming *et al*., 1999; Barros *et al*., 2001; Sweatt, 2001; Zhen *et al*., 2001; Cavallaro *et al*., 2002b).

### *Synaptic and cytoskeletal proteins*

This group includes synaptogyrin I, an abundant synaptic vesicle protein involved in short-term and long-term synaptic plasticity (Janz *et al*., 1999), and microtubule-associated protein 1B (MAP1B), which regulates the neuronal cytoskeleton during neurite outgrowth, plasticity and regeneration (Edelmann *et al*., 1996). The selective decreased expression of these genes in CA may reflect morphological adaptation of brain cells during the formation of memory.

### *Aptoptosis*

The group of proteins involved in aptoptosis includes Bcl-2 and death effector domain-containing protein. The former is increased whereas the latter is selectively decreased in CA. In agreement with other studies (Yamada *et al*., 1995) our data suggest that, beyond their roles in cell death, apoptotic and antiapoptotic cascades may play roles in synaptic plasticity.

### *Transcription regulation*

Among the group of differentially expressed genes involved in transcription is the estrogen receptor 2, which is selectively decreased in CA and is thought to mediate at least part of the complex and time-dependent effects of estrogens on memory (Rissman *et al*., 2002).

The data presented further demonstrate the utility of a cDNA microarray system as a means of dissecting the molecular basis of learning and memory. It should be emphasized that the microarray provides estimates of changes in mRNA levels which cannot be correlated with the amount and function of the gene products. Translation and post-translational modifications of many gene products and protein turnover have dramatic effects on function, and these cannot be inferred from expression analysis alone. Nevertheless, the approach used provides information on the gene expression changes which occur during learning and memory and identify molecular targets and pathways whose modulation may allow new therapeutic approaches for improving cognition (Cavallaro *et al*., 2002b).

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## Abbreviations

CA, conditioned animals; CSTA, conditioned stimulus-trained animals; LTM, long-term memory; MRGs, memory-related genes; RT-PCR, reverse transcription–polymerase chain reaction; STM, short-term memory; TGF, transforming growth factor; USTA, unconditioned stimulus-trained animals.

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