Experience-Dependent Gene Expression in the Rat Hippocampus after Spatial Learning: A Comparison of the Immediate-Early Genes Arc, c-fos, and zif268

John F. Guzowski, Barry Setlow, Edward K. Wagner, and James L. McGaugh

Neuronal immediate-early gene (IEG) expression is regulated by synaptic activity and plays an important role in the neuroplastic mechanisms critical to memory consolidation. IEGs can be divided into two functional classes: (1) regulatory transcription factors (RTFs), which can broadly influence cell function depending on the "downstream" genes they regulate, and (2) "effector" proteins, which may directly modulate specific cellular functions. The objective of the current study was to determine whether the expression of an effector IEG (Arc) was similar to, or different from, that of two well characterized RTF IEGs (c-fos and zif268) after learning. IEG RNA levels from rats trained in spatial and nonspatial water tasks were determined using RNase protection assays and in situ hybridization. Overall, the regulation of the three IEGs was similar in the hippocampus and the entorhinal and primary visual cortices. Consequently, IEG RNA levels were positively correlated within a structure. By contrast, Arc and zif268 RNA levels were not correlated or only weakly correlated across structures, although c-fos RNA levels were moderately correlated across structures. Arc RNA expression differed from that of zif268 and c-fos in two regards: (1) hippocampal Arc RNA levels were correlated with learning of the hippocampal-dependent spatial, but not hippocampal-independent cued response, water task, and (2) Arc RNA levels in the hippocampus and entorhinal cortex increased after spatial reversal learning relative to an asymptotic performance group. Thus, although the expression of Arc, zif268, and c-fos exhibited many similarities, Arc was most responsive to differences in behavioral task demands.

Key words: immediate-early; gene; spatial learning; memory; water maze; Arc; c-fos; zif268; hippocampus; entorhinal cortex; RNase protection assay; in situ hybridization

The expression of specific immediate-early genes (IEGs) is induced by neural activity that produces stable changes in synaptic strength (Abraham et al., 1993; Worley et al., 1993) and by behavioral training (Hess et al., 1995; Nagahara and Handa, 1995; Seeds et al., 1995; Vann et al., 2000). This has led to the hypothesis that IEG expression plays a role in the neuroplastic mechanisms required for memory consolidation processes (Robertson, 1992; Kaczmarek, 1993; Dragunow, 1996; Tischmeyer and Grimm, 1999). The second class encodes a diverse range of biological “effector” proteins, which have more defined and direct effects on cellular function than RTFs (Lanahan and Worley, 1998). Using subtractive hybridization techniques, Lanahan and Worley (1998) estimate that 30–40 genes encode the total neuronal IEG response and that 10–15 genes encode RTF IEGs and the rest encode effector IEGs.

Experiments using artificial synaptic stimulation demonstrate that different IEGs have different stimulus thresholds for transcriptional induction (Abraham et al., 1993; Worley et al., 1993). However, the degree to which neuronal IEGs are coordinately regulated in response to physiological stimuli is not well characterized. Furthermore, the exact nature of the IEG response to synaptic input could have distinct consequences on defined neuroplastic processes. Therefore, it will be important to determine whether IEGs are expressed coordinately as discrete subsets of the total neuronal IEGs under defined physiological conditions or whether there is a generic “IEG response.”

The activity-regulated cytoskeletal-associated (Arc) gene, also termed Arg3.1 (Link et al., 1995), is an effector IEG, the RNA and protein products of which are localized to neuronal soma and dendrites (Lyford et al., 1995). As detailed in Discussion, Arc
gene expression was most sensitive to changes in behavioral task both similarities and differences with c-fos and zif268, which have been the focus of many studies of neuroplasticity (for review, see Herrera and Robertson, 1996; Tischmeyer and Grimm, 1999). The presented findings show that the behavioral regulation of Arc RNA expression shares both similarities and differences with c-fos and zif268, but that Arc gene expression was most sensitive to changes in behavioral task demands.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (250 gm at arrival; Charles River Breeding Laboratories) were used. The rats were individually housed in a temperature- (22°C) and light-controlled vivarium (12 hr light/ dark cycle with the lights on at 7:00 A.M.), with food and water available ad libitum, and acclimatized to laboratory conditions for ~1 week before any handling or behavioral training.

Behavioral training procedures

Spatial water-task training. The apparatus used for all water tasks was a black tank (dimension 1.83 m, height 0.58 m) filled to a depth of ~20 cm with water (24 ± 2°C). For the spatial task, a submerged Plexiglas platform (20 × 25 cm; 2 cm below the surface of the water) was located at a fixed position throughout the training sessions. A training session consisted of a series of six trials with a 20 sec inter-trial interval (ITI). On each trial, the rat started from one of five positions along the side of the tank. The rat was given 60 sec to find the submerged platform. If a rat did not mount the platform within the 60 sec, it was guided to the platform by hand. The time to mount the platform was recorded as training latency for each trial. The rat was allowed to remain on the platform for 20 sec before being removed to a holding cage for the ITI.

Cued water-task training. The cued task training consisted of a series of six trials with a 40 sec ITI. Each trial consisted of the rat starting from one of five random positions along the side of the water tank. The rat was given 60 sec to find the visible platform, which was marked with a black and white striped band protruding above the surface of the water. If the rat did not mount the platform within the 60 sec, it was guided to the platform by hand. The time to mount the platform was recorded as training latency for that trial. After mounting the platform, the rat was immediately removed to a holding cage for the ITI. The visible platform was moved to different locations between each trial, so that the rat's starting position and the platform location were unique between trials.

Spatial reversal water-task training. Rats were trained in the spatial water maze (see above) over 3 d with the submerged platform located in one fixed position. On each day, the rats received two training sessions of six trials per session, separated by 30 min. On the final experimental day, one group (seven sessions; n = 6) received training exactly as on the six previous sessions. For a second group (seven session reversal; n = 6), the submerged platform was moved to a new location in the pool; these rats were familiar with the task demands but had to learn the new spatial location of the platform. A third group was also included in this experiment (first session; n = 6); these rats were trained in the spatial water task for the first time. To minimize the stress on the seventh session reversal and other two groups, all rats were placed on the platform for 10 sec before the start of each training session for this experiment.

RNA detection methods

Brain areas analyzed. The dorsal hippocampus (approximately ~3.6 mm from bregma (Paxinos and Watson, 1986)) was the focus of the current study because this region has been shown to be required for acquisition and consolidation of learning for the spatial water task (E. Moser et al., 1993; M. B. Moser et al., 1995; Guzowski and McGaugh, 1997; Guzowski et al., 2000). IEG RNA levels in the primary visual cortex and lateral entorhinal cortex [both at approximately ~6.0 mm from bregma (Paxinos and Watson, 1986)] were also examined in one experiment. The entorhinal cortex was examined because it provides a major input to the hippocampus (for review, see Johnson and Amaral, 1998) and is important for spatial learning (Nagahara et al., 1995; Cho and Kesner, 1996). The primary visual cortex was examined to compare IEG regulation in a primary sensory cortical area (engaged during task performance) to areas of higher order and polymodal processing (i.e., the hippocampus and entorhinal cortex).

Tissue dissection and RNA extraction for RNase protection assays. Water task-trained and caged control rats were anesthetized with sodium pentobarbital, transcardially perfused with 0.1 M phosphate buffer, and decapitated. The brains were removed rapidly, and 1-mm-thick coronal sections were prepared using a tissue matrix (Ted Pella Instruments). The sections were frozen on dry ice, and hippocampal and cortical tissue was removed using a punch technique (Palkovits and Brownstein, 1988) with a glass pipette (inner diameter 1.0 mm). Single or multiple punches were taken to remove the region of interest. Hippocampal punches removed the entire hippocampus and dentate gyrus. Punches of the primary visual or entorhinal cortices removed all neuronal layers within that region of cortex. Punches from both hemispheres were pooled for a given structure for each rat. Total RNA was prepared using TRIZol according to the manufacturer’s instructions (Life Technologies-BRL). RNA concentrations were determined spectrophotometrically, and RNA aliquots were stored at −70°C until RNase protection assay (RPA) analysis.

Preparation of brain sections for fluorescent in situ hybridization. Thirty minutes after training, the rats were anesthetized with sodium pentobarbital and decapitated. The brains were removed rapidly, flash frozen in isopentane equilibrated in a dry ice/ethanol slurry, and stored at −70°C for further processing. Coronal brain sections (2 mm thick) were prepared with a tissue matrix and used to create tissue blocks. Sections containing the dorsal hippocampus from one caged control rat and one water task-trained rat were placed in a plastic mold, covered with OCT media (VWR), and frozen on dry ice. This was repeated for the other caged control and water task-trained rats. All remaining blocks (8 µm thick) were prepared using a cryostat and collected on Superfrost Plus slides (VWR). Slides were stored at ~70°C until processing for fluorescent in situ hybridization (FISH).

Riboprobe preparation. For the RNase protection assays, 32P-labeled actin, c-fos, and zif268 antisense riboprobes were prepared from commercially available plasmid templates (Ambion, Austin, TX). The riboprobe was generated from a modified Arc cDNA plasmid. A nearly full-length cDNA pBluescript clone (Lyford et al., 1995) was restricted with XhoI and NdeI, treated with Klenow enzyme to blunt the DNA ends, and closed with T4 DNA ligase. These steps removed all but 195 bases of Arc 5' sequence from bases 33–226 of the published sequence (GenBank accession number U19866). The resulting plasmid was treated with XhoI to generate a linearized template for the synthesis of the Arc antisense riboprobe. Radioactively labeled riboprobes were generated by in vitro transcription with T7 or T3 RNA polymerases (Maxscript kits; Ambion) and α-[32P]UTP (Amersham Pharmacia; 800 Ci/mmol) at 15°C. Unlabeled UTP was added during the synthesis of the actin riboprobe but was not added during the synthesis of the IEG riboprobes. The unlabeled UTP lowered the specific activity of actin riboprobes 25-fold relative to the IEG riboprobes. Digoxigenin-labeled riboprobes were generated by in vitro transcription with T7 (antisense) or T3 (sense) RNA polymerases (Maxiscript kit; Ambion) and digoxigenin RNA labeling mix (Roche Molecular Biochemicals). Riboprobes were purified on G-50 spin columns (Amersham Pharmacia, Arlington Heights, IL). Antisense and sense Arc riboprobes for FISH were prepared from a nearly full-length cDNA pBluescript clone (Lyford et al., 1995), as described previously (Guzowski et al., 1999). The plasmid was treated with Xbal to generate the linearized template for the antisense riboprobe and with XhoI to generate the linearized template for the sense riboprobe. Digoxigenin-labeled riboprobes were generated by in vitro transcription with T7 (antisense) or T3 (sense) RNA polymerases (Maxiscript kit; Ambion) and digoxigenin RNA labeling mix (Roche Molecular Biochemicals). Riboprobes were purified on G-50 spin columns (Amersham Pharmacia).
polyacrylamide gels containing 8 M urea with the full-length probes and 32P-labeled DNA markers (HindII digested χX714 DNA; Promega, Madison, WI).  

Quantifying RNA levels by RNase protection assay. RPA gels were dried on Whatman 3mm paper. Dried gels were exposed to a phosphor screen for 16–36 hr and scanned using a PhosphorImager 445Si (Molecular Dynamics). Volume analysis was performed using ImageQuant software (version 1.1; Molecular Dynamics). Data values were obtained for each IEG RNA band and for the actin band, which served as an internal control. IEG values were normalized to actin values for each sample.  

Fluorescent in situ hybridization. Fluorescent in situ hybridization (FISH) was performed on slide-mounted brain sections using a digoxigenin-labeled Arc antisense riboprobe as described in detail elsewhere (Guzowski et al., 1999). Arc riboprobe was detected with an antidigoxigenin–horseradish peroxidase conjugate (Roche Molecular Biochemicals) and the TSA-Direct Cyanine-3 kit (NEN Life Sciences). Nuclei were counterstained with YOYO-1 (Molecular Probes), and slides were mounted with an anti-fade medium (Vectorshield; Vector Laboratories, Burlingame, CA). Four slides containing the dorsal hippocampus (approximately ~3.6 mm from bregma) were analyzed for each rat. Images (1024 × 1024 pixels) were acquired using a Leica TCS-4D laser scanning confocal microscope with a krypton/argon laser. A 10× objective was used for analysis of CA1 and CA3 regions. The field of view, using this objective was 1000 × 1000 μm. Confocal microscope settings (pinhole settings, and voltage settings and offset) were carefully adjusted to ensure that the full grayscale range was used and to minimize the occurrence of saturated pixels. RGB TIFF images were analyzed in Adobe Photoshop 5.5. First, the pyramidal cell layer was selected by visualizing only the color channel containing the YOYO-1 information and using the “Lasso” tool. Then, the mean pixel density value for the visualizing only the color channel containing the YOYO-1 information. (Arc RNA staining). Finally, the values for the trained rats were normalized using the values from the caged controls. This normalization procedure minimizes artifact caused by slide-to-slide variation in signal intensity and background.  

For analysis of Arc-positive cells in the dentate gyrus granule cell layer, a modified approach was taken because of the different pattern of Arc staining observed in the dentate. Overall, a much lower percentage of dentate gyrus granule cells express Arc as compared with CA1 and CA3 neurons (Guzowski et al., 1999). Because of this low density of Arc-positive cells in the dentate, it is more informative to count Arc-positive cells. Images were acquired with the same confocal microscope, but with a 5× objective, which yielded a field of view of 2000 × 2000 μm. Arc-positive cells were counted for each field. The total number of Arc-positive cells was determined for all four slides for each trained rat and each control rat. As done for analysis of CA1 and CA3 regions, the value obtained for each trained rat was normalized to the value for the control rat in that tissue block (see Preparation of brain sections for fluorescent in situ hybridization, above).  

Statistics  

For behavioral data, ANOVA or repeated measures ANOVA was used to analyze individual trials or trial sessions, respectively. Fischer’s post hoc tests were used for pairwise comparisons. In some experiments, the IEG RNA data violated a principal assumption of parametric statistical tests. Specifically, the variance between groups was often different as determined using an equality of variances F test. For this reason, non-parametric tests were used in the analysis of the RNA data. For comparisons among three or more groups, the Kruskal–Wallis test was used; if α < 0.05, then pairwise comparisons were made using the Mann–Whitney U test. For correlational analyses, the Spearman correlation coefficient, R_s, was determined. In all instances, a probability level of <0.05 was accepted as statistically significant.

RESULTS  

Arc, zif268, and c-fos RNA levels are rapidly and transiently increased in the dorsal hippocampus by spatial water task training  

To quantify changes in IEG RNA levels from brain RNA samples, a multiple probe RNase protection assay for the simultaneous detection of Arc, c-fos, and zif268 mRNAs was developed and used for most of the experiments described here. In addition to the IEG riboprobes, a low specific activity riboprobe for β-actin RNA was included in the RPA to normalize IEG signals between samples. The rapid induction of Arc, c-fos, and zif268 RNAs by neural activity was demonstrated in rats treated with the convulsant pentylentetrazole (50 mg/kg, i.p.), which induced behavioral seizures. The rats were killed 45 min after the onset of seizures, hippocampi were removed, and total RNA was prepared. As expected (Morgan et al., 1987; Saffen et al., 1988; Lyford et al., 1995), hippocampal IEG RNA levels were increased by seizure activity relative to controls (Fig. 1a). RPA autoradiographs of RNA from the hippocampi and the visual cortices of rats trained in different spatial water tasks demonstrate the utility of the RPA for behavioral studies (Fig. 1b); these data are part of a larger experiment shown in Figures 4 and 5 and Tables 2 and 3.  

To investigate the regulation of Arc, c-fos, and zif268 by limited behavioral training, rats were trained in a single session of the spatial water task. The dorsal hippocampus was the focus of all of the current experiments because lesion studies have revealed this region to be critical for spatial water-task acquisition (E. Moser et al., 1993; M. B. Moser et al., 1995). Moreover, transient disruption of CREB (Guzowski and McGaugh, 1997) or Arc (Guzowski et al., 2000) protein expression in the dorsal hippocampus impairs long-term memory for spatial water-task training without affecting task acquisition or short-term memory. The rats were given six trials to learn the location of the submerged platform. Training lasted for ~8–10 min for each rat. Task performance improved significantly during the training session (data not shown; p < 0.0001, repeated measures ANOVA). At 0.5, 2, or 6 hr after training, the rats were killed, and tissue was dissected from the dorsal hippocampus. Session training performance was not different between rats in any of the time point groups (data not shown; p = 0.85, repeated measures ANOVA). Hippocampal IEG RNA levels from the trained rats and caged control rats were analyzed by RPA. Levels of all three IEG RNAs were significantly higher than caged controls at 0.5 hr, but not 2 or 6 hr, after training (Fig. 2) (p < 0.05, for each Mann–Whitney U test).  

Hippocampal IEG RNA expression of rats trained in either the spatial or cued water task  

Separate groups of rats were trained in either the spatial or cued water tasks. Task acquisition, as measured by latency to mount either the visible or the submerged platforms, was similar between the two groups (Fig. 3A) (p = 0.24, repeated measures ANOVA). The rats were killed 30 min after training, and hippocampal IEG RNA levels were quantified by RPA.  

Arc, zif268, and c-fos RNA levels from the dorsal hippocampus of rats trained in both water tasks were significantly higher than those from caged control rats (Fig. 3B) (p < 0.001 for each Mann–Whitney U test). However, no group differences in hippocampal IEG RNA levels were observed between rats trained in the two different water tasks (Fig. 3B) (p > 0.45 for each Mann–Whitney U test).  

The relationship between hippocampal IEG RNA levels and rate of learning in rats trained in the two tasks was also examined (Fig. 3C, Table 1). The rats’ performance in the spatial task quickly improved in the first three trials and stabilized on the last three trials (Fig. 3A). Therefore, performance on the last three trials provides an appropriate measure of spatial learning ability for individual rats. In rats trained in the spatial task, a significant negative correlation was observed between Arc RNA levels and mean escape latency on the last three trials of the training session (Fig. 3C, Table 1) (R_s = −0.76; p < 0.05): the best spatial learners tended to have the highest levels of hippocampal Arc gene ex-
expression. Although zif268 and c-fos were also negatively correlated with learning in the spatial task, these correlations did not reach statistical significance. By contrast, there was no correlation between IEG RNA levels and escape latency on the final three trials of cued-task trained rats, nor was there a correlation between IEG RNA levels and mean escape latency for all six trials of either the spatial or cued tasks (Table 1).

Arc RNA expression in the hippocampus and entorhinal cortex is increased by spatial reversal training

Separate groups of rats were trained in one of three variations of the spatial water task (Fig. 4, 1st session, 7th session, or 7th session-reversal) (see Materials and Methods for training details of the individual groups). Each group consisted of six rats. Trained rats were killed 30 min after the final training trial, and an equal number of rats were killed directly from their home cages to determine baseline levels of IEG expression. RNA was prepared from dissected tissue (dorsal hippocampus, entorhinal cortex, and primary visual cortex) of the trained rats and caged control rats and was analyzed by RPA.

The seventh session group performed the familiar task at an asymptotic level of performance (Fig. 4B). The seventh session-reversal group learned the new platform location in one trial and performed at levels similar to the seventh session group for the remaining five training trials. The first session group learned the task rapidly and performed at the level of the other two groups by the fifth and sixth trials.

IEG RNA levels for each of the trained groups were significantly higher than those of the caged controls in the examined brain regions (Fig. 5). In the dorsal hippocampus, RNA levels for all IEGs were significantly lower in the seventh session group as compared with the first session group (Fig. 5A). Arc RNA levels of the seventh session-reversal group were significantly higher than those of the first session group (Fig. 5A). The behavioral expression profile for c-fos RNA was similar in the dorsal hippocampus, entorhinal cortex, and primary visual cortex (Fig. 5). In each structure, c-fos RNA levels were highest in the first session group, but did not differ significantly between the seventh session and the seventh session-reversal groups. In short, the pattern was as follows: caged < seventh session = seventh session reversal < first session. This pattern was also observed for zif268 RNA expression in the hippocampus (Fig. 5A).

Arc and zif268 RNA expression in the entorhinal (Fig. 5B) and visual (Fig. 5C) cortices exhibited both similarities and differences compared with that observed in the hippocampus. In the entorhinal cortex, as in the hippocampus, Arc RNA levels of the seventh session-reversal group were significantly higher than those of the seventh session group, but not different from those of the first session group (Fig. 5B). By contrast, Arc RNA levels did...
not differ significantly in the visual cortex for any of the water task-trained groups (Fig. 5C). Unlike the hippocampus, zif268 RNA levels in the entorhinal and visual cortices did not differ significantly among any of the trained groups (Fig. 5B, C).

We also compared the levels of different IEGs within a structure (Table 2) or across structures (Table 3). In both analyses, we did not include the caged control animals. The low IEG RNA levels of the controls tended to create two clusters of points, one for the caged animals and one for the trained animals, which created high, but inconsequential, correlation coefficients. Furthermore, we did not analyze the different behavioral groups separately; the limited group size (six rats per group) precluded a rigorous analysis. For the most part, IEG RNA levels in the dorsal hippocampus were modestly correlated for the trained rats (Table 2). By contrast, the IEG RNA levels were more strongly correlated in the entorhinal and visual cortices. Across different brain structures, Arc or zif268 RNA levels were only weakly correlated, if at all, and c-fos RNA levels were modestly correlated (Table 3).

Spatial water-task training increases Arc RNA expression in the hippocampal pyramidal cell layers and in the dentate gyrus granule cell layer

To determine whether changes in Arc gene expression were limited to specific subregions of the hippocampus, we performed fluorescent in situ hybridization using Arc antisense riboprobe and brain sections from caged control rats and rats killed 30 min after spatial water-task training. Four sections containing the dorsal hippocampus were analyzed for each rat. For each specific region, no differences in Arc RNA expression were observed between left and right hemispheres for either the caged or trained groups. Consequently, data from both hemispheres were combined for each animal in subsequent analyses. Spatial water-task

### Table 1. Correlations between behavioral measures and hippocampal IEG RNA levels

<table>
<thead>
<tr>
<th>Measure</th>
<th>IEG RNA</th>
<th>Spatial task</th>
<th>Cued task</th>
<th>Both tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last three trials</td>
<td>Arc</td>
<td>−0.76</td>
<td>0.18</td>
<td>−0.32</td>
</tr>
<tr>
<td></td>
<td>zif268</td>
<td>−0.58</td>
<td>0.13</td>
<td>−0.14</td>
</tr>
<tr>
<td></td>
<td>c-fos</td>
<td>−0.49</td>
<td>0.12</td>
<td>−0.09</td>
</tr>
<tr>
<td>All six trials</td>
<td>Arc</td>
<td>−0.55</td>
<td>−0.21</td>
<td>−0.26</td>
</tr>
<tr>
<td></td>
<td>zif268</td>
<td>−0.36</td>
<td>0.22</td>
<td>−0.03</td>
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<tr>
<td></td>
<td>c-fos</td>
<td>−0.26</td>
<td>0.39</td>
<td>0.10</td>
</tr>
</tbody>
</table>

The Spearman correlation coefficients are given; the comparison significant at \( p < 0.05 \) is indicated in bold font.

Figure 3. Hippocampal IEG RNA expression after spatial and cued water-task training. Rats were trained in either the spatial or cued water tasks and killed 30 min later. IEG RNA levels from dorsal hippocampus were analyzed by RPA. A. Acquisition data for rats trained in each task. Performance, as measured by latency to mount the hidden (spatial) or visible (cued) platform, was not different between the two groups (\( p = 0.24 \), repeated measures ANOVA). B. Hippocampal IEG RNA levels for rats trained in both tasks were significantly above caged control levels (\( p < 0.001 \), Mann–Whitney U test) but were not different from each other. C, D. Negative correlation between hippocampal Arc RNA levels and performance in the spatial (C), but not cued (D), water task. See Results for additional information. The Spearman correlation coefficient (\( R_s \)) is indicated for both cued and spatial trained groups (*\( p < 0.05 \)). \( n = 8–9 \) rats per group.
correlation between hippocampal and entorhinal cortices increased in rats that learned a new platform location relative to those performing a familiar task (Fig. 5), (3) IEG RNA levels were transiently increased after spatial learning (Fig. 2), which were consistent with other studies (Tischmeyer et al., 1990; Grimm and Tischmeyer, 1997). It is not clear why hippocampal c-fos RNA levels were below control levels 6 hr after training.

Arc gene expression was compared directly with that of c-fos in one other behavioral study. Montag-Sallaz and colleagues (1999) reported several differences in the time course and regional pattern of Arc and c-fos induction by gustatory novelty. Although the differences between this study and our findings are not easily reconciled, it is possible that under very different behavioral conditions (gustatory novelty in the absence of specific task demands and spatial learning), distinct regional patterns of IEG expression may emerge.

IEG expression and hippocampal function
The similar levels of IEG expression of rats trained in either spatial or cued water tasks indicates that both tasks led to similar activation of signaling/transcriptional pathways in hippocampal neurons. This finding is consistent with electrophysiological recording studies indicating that hippocampal neurons encode both spatial and nonspatial information (Young et al., 1994; Markus et al., 1995; Gothard et al., 1996; Wood et al., 1999). Additionally, behavioral studies demonstrate that the hippocampus encodes spatial information even when animals are trained in tasks that do not explicitly require its use (Packard and McGaugh, 1996), including the cued water task (Rapp et al., 1987).

Despite the lack of an overall group effect, the correlation between hippocampal Arc RNA levels and task performance in the spatial, but not cued, water task (Fig. 3, Table 1) suggests that...
Arc gene expression is related to task-relevant encoding processes. In this view, cued task-trained rats processed much of the same spatial and nonspatial information as spatial task-trained rats, but this information was not required for solving the cued task. By contrast, information processed by the hippocampus was necessary for solving the spatial task, and consequently, rats that had a greater activation of hippocampal neurons (as indicated by Arc expression) tended to perform better in the spatial task. This interpretation is consistent with findings from lesion studies: hippocampal lesions impair performance in the spatial, but not cued, water task (Morris et al., 1982).

In the current studies, hippocampal IEG expression decreased modestly with extended training but remained well above caged control levels (Fig. 5A). Similarly, it was demonstrated recently that retrieval of contextual fear conditioning resulted in increased zif268 RNA expression in CA1 neurons (Hall et al., 2001). The lack of habituation of the IEG response, although the measured behavioral response (escape latency) was asymptotic, suggests that retrieval engages some of the same cellular processes activated during initial acquisition. In this view, signal transduction pathways governing IEG transcription in hippocampal neurons do not discriminate between the processing and use of new information as compared with that of familiar information. Certainly, firing activity patterns of hippocampal neurons are not obviously different in familiar and novel environments (O’Keefe, 1976; Muller and Kubie, 1987), and “place fields” of hippocampal neurons appear during an initial exposure to an environment and

Table 2. Correlations between IEG RNA levels within a structure

<table>
<thead>
<tr>
<th>Structure</th>
<th>IEG 1</th>
<th>IEG 2</th>
<th>R_s value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>Arc</td>
<td>zif268</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Arc</td>
<td>c-fos</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>zif268</td>
<td>c-fos</td>
<td>0.55</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>Arc</td>
<td>zif268</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Arc</td>
<td>c-fos</td>
<td>0.74</td>
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<tr>
<td></td>
<td>zif268</td>
<td>c-fos</td>
<td>0.64</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>Arc</td>
<td>zif268</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Arc</td>
<td>c-fos</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>zif268</td>
<td>c-fos</td>
<td>0.75</td>
</tr>
</tbody>
</table>

The Spearman correlation coefficients are given; comparisons significant at p < 0.05 are indicated in bold font. The above values were calculated for all of the trained rats of Figures 4 and 5 (n = 18 rats); the caged control rats were not included in the analyses. See Results for details.

Arc gene expression is related to task-relevant encoding processes. In this view, cued task-trained rats processed much of the same spatial and nonspatial information as spatial task-trained rats, but this information was not required for solving the cued task. By contrast, information processed by the hippocampus was necessary for solving the spatial task, and consequently, rats that had a greater activation of hippocampal neurons (as indicated by Arc expression) tended to perform better in the spatial task. This interpretation is consistent with findings from lesion studies: hippocampal lesions impair performance in the spatial, but not cued, water task (Morris et al., 1982).

In the current studies, hippocampal IEG expression decreased modestly with extended training but remained well above caged control levels (Fig. 5A). Similarly, it was demonstrated recently that retrieval of contextual fear conditioning resulted in increased zif268 RNA expression in CA1 neurons (Hall et al., 2001). The lack of habituation of the IEG response, although the measured behavioral response (escape latency) was asymptotic, suggests that retrieval engages some of the same cellular processes activated during initial acquisition. In this view, signal transduction pathways governing IEG transcription in hippocampal neurons do not discriminate between the processing and use of new information as compared with that of familiar information. Certainly, firing activity patterns of hippocampal neurons are not obviously different in familiar and novel environments (O’Keefe, 1976; Muller and Kubie, 1987), and “place fields” of hippocampal neurons appear during an initial exposure to an environment and

Table 3. Correlations between IEG RNA levels across brain structures

<table>
<thead>
<tr>
<th>Structure 1</th>
<th>Structure 2</th>
<th>IEG RNA</th>
<th>R_s value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>Entorhinal cortex</td>
<td>Arc</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>zif268</td>
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<tr>
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<td>c-fos</td>
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<td>Visual cortex</td>
<td>Arc</td>
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<td>Arc</td>
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</tr>
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<td></td>
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<td>c-fos</td>
<td>0.81</td>
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The Spearman correlation coefficients are given; comparisons significant at p < 0.05 are indicated in bold font. The above values were calculated for all of the trained rats of Figures 4 and 5 (n = 18 rats); the caged control rats were not included in the analyses.
Figure 6. Increase in Arc RNA expression in the CA1 pyramidal cell layer 30 min after spatial water-task training. a and c show nuclei (blue) and Arc RNA staining (red) for a caged control and a spatial water-task trained rat, respectively. b and d show grayscale images of Arc RNA staining from a and c, respectively. Final magnification is 40×. Scale bars (shown in a and c): 50 μm. See Materials and Methods for experimental details and Table 4 for the summary of the quantitative analyses.

Figure 7. Increase in Arc RNA expression in the dentate gyrus and CA3 regions 30 min after spatial water task training. a and c show nuclei (blue) and Arc RNA staining (red) for a caged control and a spatial water task-trained rat, respectively. b and d show grayscale images of Arc RNA staining from a and c, respectively. Final magnification is 5×. Scale bars (shown in a and c): 200 μm. See Materials and Methods for experimental details and Table 4 for the summary of the quantitative analyses.
gyrus of rats trained in the spatial water task. 

The role of experience-dependent Arc gene expression in synaptic plasticity

The current findings add to a growing body of evidence indicating that Arc plays a critical role in synaptic plasticity. Arc is the only known IEG in which both RNA and protein products are localized to dendrites (Link et al., 1995; Lyford et al., 1995; Wallace et al., 1998). Arc RNA expression is regulated by patterned stimulation that induces long-term potentiation (Lyford et al., 1995) and by physiological neural activity (Guzowski et al., 1999; Montag-Sallaz et al., 1999; Guthrie et al., 2000). Furthermore, the dendritic localization of Arc RNA and protein can be regulated by synaptic activity (Steward et al., 1998). These properties suggest that Arc may be subject to multiple levels of regulation (intracellular trafficking, local regulation of translation at defined synapses, etc.) and may make Arc a unique, rather than prototypical, effector IEG. Although the cellular function of Arc is not known at this time, Arc protein interacts with structural proteins (Lyford et al., 1995) and calcium and calmodulin-dependent kinase II (CaMKII) (P. F. Worley, personal communication). CaMKII is a dendritic protein critical to synaptic plasticity and long-term memory formation (Mayford et al., 1995, 1996). Therefore, Arc may affect synaptic plasticity by modulating the activity, intracellular trafficking, or substrate specificity of CAMKII.

The above findings are consistent with the hypothesis that Arc may mediate neuroplastic changes at discrete synapses necessary for memory consolidation processes. In support of this view, temporary inhibition of hippocampal Arc protein expression impairs the maintenance of long-term potentiation, without affecting its induction, and impairs consolidation of long-term memory for spatial water-task training, without affecting task acquisition or short-term memory (Guzowski et al., 2000). Furthermore, Arc expression in CA1 neurons is specifically induced by neural encoding processes, as determined using a novel FISH approach that provides both temporal and cellular resolution (Guzowski et al., 1999). These interesting properties indicate that continued studies into the regulation and function of Arc will likely provide new insights into the molecular and cellular bases of cognition.

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Table 4. Increase in Arc RNA expression in CA1, CA3, and dentate gyrus of rats trained in the spatial water task

<table>
<thead>
<tr>
<th>Region</th>
<th>Relative Arc expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentate gyrus</td>
<td>150 ± 9</td>
</tr>
<tr>
<td>CA1</td>
<td>261 ± 33</td>
</tr>
<tr>
<td>CA3</td>
<td>200 ± 36</td>
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</table>

The reported percentages are given relative to caged control rats. Four sections containing the dorsal hippocampus and processed for Arc RNA signal using FISH were analyzed for each rat. For CA1 and CA3 regions, Arc fluorescent intensity was determined over the appropriate region of the pyramidal cell layer. For the dentate gyrus, Arc RNA-positive cells in the granule cell layer were counted. The total mean number of Arc-positive dentate cells per rat was 92 for the caged control group and 138 for the water task-trained group. See Materials and Methods for details on the FISH and normalization procedures. n = 3 rats per group.

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REFERENCES


Markus EJ, Qin YL, Leonard B, Skaggs WE, McNaughton BL, Barnes CA (1995) Interactions between location and task affect the spatial