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Spatial behavior and seizure-induced changes in *c-fos* mRNA expression in young and old rats

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Abstract

The subcellular processes of gene induction and expression in the hippocampus are likely to underlie some of the known age-related impairments in spatial learning and memory. It is well established that immediate-early genes are rapidly and transiently induced in response to neuronal activity and this expression is required for stabilization of durable memories. To examine whether age-related memory impairment might be caused, in part, by differences in the level of cellular activation or subcellular processing, *c-fos* expression in CA1 pyramidal and dentate gyrus granule cells in the dorsal hippocampus of young and old rats was determined using fluorescence in situ hybridization and reverse transcription polymerase chain reaction. No significant age differences were found in the numbers of pyramidal or granule cells that show *c-fos* expression; however, *c-fos* mRNA transcripts were altered in these 2 cell types in aged animals. These findings suggest that though the networks of cells that participate in behavior or seizure-induced activity are largely maintained in aged rats, their RNA transcript levels are altered. This might, in part, contribute to cognitive deficits frequently observed with advancing age.

Keywords

Aging; Immediate early-genes; Transcription; Learning and memory; Synaptic plasticity; Seizures

1. Introduction

Transcription factors encoded by immediate-early genes (IEGs) play an important role in neuronal plasticity. The IEG *c-fos* is rapidly and transiently induced in response to physiological stimuli (e.g., Clayton, 2000; Curran and Morgan, 1987; Herdegen and Leah, 1998; Lanahan and Worley, 1998; Tischmeyer and Grimm, 1999) and has been used as a

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Animal handling and procedures were in accordance with the National Institutes of Health guidelines and the University of Arizona's Institutional Animal Care and Use Committee.

Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2012.10.017>.

marker for neuronal activity (Abraham et al., 1991; Burke and Barnes, 2006; Cole et al., 1989; Dragunow et al., 1989; Jones et al., 2001; Lanahan et al., 1997; Loebrich and Nedivi, 2009; Qian et al., 1993; Worley et al., 1993). Blocking expression of IEGs has been shown to impair memory consolidation (Guzowski et al., 2000; Jones et al., 2001; Plath et al., 2006). The 2 classes of genes that are activated as a result of synaptic activity are either transcriptional regulators that alter other target genes, or are direct effector genes, including structural proteins, signaling enzymes, and growth factors. The IEG *c-fos* is a transcription regulator that is rapidly induced in the brain by direct stimulation, seizure (Kaczmarek and Nikolajew, 1990; Silva and Giese, 1994) or by behavioral activity such as spatial exploration (Qiang et al., 1999; Santin et al., 2003; Teather et al., 2005).

Because IEG expression plays a crucial role in plasticity and memory mechanisms, it has been important to determine the extent to which changes in the pathways that engage IEGs are altered during aging (Blalock et al., 2003; Brightwell et al., 2004; Bucci et al., 1998; Jiang et al., 1989, 1997; Marrone et al., 2008; Penner et al., 2011; Rowe et al., 2007; Schmoll et al., 2005; Small et al., 2004; Smith et al., 2001). One of the first studies to investigate the expression of *c-fos* in the aged brain used long-term potentiation (LTP)-inducing stimulation to activate its expression in young and old rats (Worley et al., 1993). With the methods available at that time (autoradiography) no age differences were detected in *c-fos* expression in hippocampal granule cells. Similarly, a later study by Bucci et al. (1998) found no differences in *c-fos* protein (CA1 subregion) or mRNA expression (measured by optical densitometry in the hippocampus) after treatment with the cholinergic agonist pilocarpine (Bucci et al., 1998). Using a sensitive reverse Northern blot strategy for quantification of *c-fos* expression, Lanahan et al. (1997) detected differences between young and old *c-fos* RNA levels; however, the levels were increased in the aged versus adult hippocampus after maximal electroconvulsive shock (MECS) or LTP-inducing stimulation. The apparent discrepancies in these results might be because of the methods used to induce and to detect *c-fos* expression. The studies that have investigated changes in basal or constitutively-expressed levels of *c-fos*, rather than activated expression, have not revealed age-related differences (Desjardins et al., 1997; Smith et al., 2001), although the sensitivity of the methods used might have contributed to these findings. To resolve these discrepancies, quantitative methods were applied to measure cell-specific expression and transcription of *c-fos* in young and aged hippocampus after behavior or seizure-induced stimulation. The combination of analytical tools used allow quantitative region-selective assessment of the transcript in 1 hemisphere with individual single-cell identification of behaviorally relevant circuits in the other hemisphere of the same brain.

2. Methods

2.1. Animals

Male (young: 9-month-old, $n = 41$; old: 24 months old, $n = 41$) Fisher 344 rats were housed individually and maintained on a 12-hour light/dark cycle. Animals were handled before the spatial behavioral experience to ensure minimal stress during the actual experiment. Animal handling and procedures were in accordance with the National Institutes of Health guidelines and the University of Arizona's Institutional Animal Care and Use Committee.

2.2. Behavioral procedures

Animals assigned to behavioral conditions were screened on the Morris swim task for their spatial and visual discrimination ability as described previously by Shen and Barnes (1996). Rats performed 6 spatial trials for 4 consecutive days (with a hidden platform), and 6 visual trials on 2 consecutive days in which the rats could see the marked platform. Behavior was monitored by an overhead video camera and a tracking unit (VP114; HVS Image). Young

and old rats were then divided into the following 6 groups: (1) rats that explored a novel environment for 5 minutes and were then sacrificed ($n = 12$); (2) rats that explored environment A for 5 minutes, were allowed to rest 20 minutes in their home cage, and then allowed to forage for another 5 minutes (A-20'-A group; $n = 12$); (3) rats that were given MECS and sacrificed 5 minutes later ($n = 12$); (4) rats that were given MECS and sacrificed 30 minutes later ($n = 8$); (5) rats that were given MECS and sacrificed 60 minutes later ($n = 10$); and, (6) rats that did not explore the environment but were taken directly from their home cage for sacrifice (caged control group; $n = 28$), serving as the negative control for all treatment groups. A schematic of behavioral procedures used is shown in Supplemental Figure 1.

The first and A-20'-A groups explored a novel environment consisting of a $60 \times 60 \text{ cm}^2$ platform surrounded by 30 cm high walls, located in a room with unique distal cues and lighting conditions. The platform was divided into nine 400 cm^2 grids. Uniform and complete exposure to the environment was accomplished by picking up the animal and releasing it into the center of 1 of the grids on a pseudo-random schedule every 15 seconds during the 5-minute exploration period. The groups that were administered MECS treatment were given a 1-second, 100-Hz, 85-mA stimulus of a 0.5 msec square wave pulse delivered using a UGO Basile ECT unit Model 7801 (Comerio, Italy) (Cole et al., 1990). These animals were carried to the experimental room in towel-lined pots and given electrically induced seizures immediately after attachment of saline-soaked ear clips. Recovery from the treatment typically occurred within 1-2 minutes for all the animals.

2.3. Brain extraction and dissection

After the various treatments, rats were anesthetized with 5% isoflurane and decapitated with a rodent guillotine. Brains were rapidly removed, hemisected and 1 half quickly frozen in isopentane cooled over an ethanol/dry ice bath and stored at -70°C until sectioning for in situ hybridization. The second half of the brain was used for reverse transcription (RT) polymerase chain reaction (PCR) after the CA1 and dentate gyrus (DG) subregions were dissected from the rest of the brain as described in Wenk and Barnes (2000). Although the dissected CA1 subregion could have included a small number of CA3 pyramidal cells, most of the cells were from CA1 and therefore will be referred to as CA1 subregion throughout this report.

2.4. Fluorescence in situ hybridization

Hemisectons containing the dorsal hippocampus approximately -3.2 to -3.8 mm from Bregma (Paxinos and Watson, 1986) were placed in plastic molds and covered with Tissue-Tek OCT compound so that all experimental conditions were represented in each block for each time point. Use of this technique minimizes slide-to-slide variation in signal detection. Twenty μm thick coronal sections were cut and arranged on each slide. Sections were thawmounted and dried before storage at -70°C . Riboprobes were generated from the rat *c-fos* cDNA (approximately 1.8 kb), kindly provided by Dr Tom Curran, using a commercial RNA transcription kit (Maxiscript; Ambion, Austin, TX, USA) and RNA labeling nucleotide mix containing digoxigenin or fluorescein-tagged UTP Uridine-5'-triphosphate (Roche Molecular Biochemicals, Nutley, NJ, USA). Fluorescence in situ hybridization was performed as described previously (Guzowski et al., 1999). Briefly, riboprobes were purified on a G-50 spin column (Boehringer Mannheim, Indianapolis, IN, USA) and a small aliquot subjected to gel electrophoresis before use for determination of yield and integrity of the riboprobes. Slides containing the sections were thawed to room temperature, fixed with freshly prepared buffered 4% paraformaldehyde, treated with 0.5% acetic anhydride/1.5% triethanolamine, incubated in methanol and acetone (1:1) for 5 minutes and equilibrated in 2X SSC (saline-sodium citrate). Sections were incubated with 100 μL 1X prehybridization

buffer (Sigma, St. Louis, MO, USA) for 30 minutes at room temperature. Approximately 100 ng of riboprobe was diluted in 1X enhanced hybridization buffer (Amersham, Piscataway, NJ, USA), heat denatured at 90 °C, chilled on ice, and applied to each section. A cover slip was placed on each slide and slides were incubated overnight at 56 °C. Posthybridization washes started with 2X SSC, and increased in stringency to 0.5X SSC at 56 °C. RNase A (10 µg/mL) at 37 °C was used to degrade any single-stranded RNA. After quenching the endogenous peroxidases with 2% H₂O₂, slides were blocked with NEN blocking agent (PerkinElmer, Boston, MA, USA) and incubated with an anti-digoxigenin or anti-fluorescein antibody conjugated with HRP (Roche Molecular Biochemicals) overnight at 4 °C. Slides were then washed with Tris-buffered saline containing 0.05% Tween-20 and the HRP-antibody conjugate detected using a Cyanine-3 (CY3) tyramide signal amplification kit (PerkinElmer). After counterstaining with SYTOX (Molecular Probes, Eugene, OR, USA) cover slips were applied to the slides with a small amount of Vectashield antifade media (Vector Labs, Burlingame, CA, USA) and sealed with nail polish.

2.5. Confocal microscopy and cellular analysis

Stained sections were imaged using a Zeiss 510 Metaseries laser confocal microscope with a Plan Apo 40× oil immersion objective, numerical aperture 1.3, or a Plan Neofluar 10× objective. Laser settings, detector gain, and offsets were kept constant after initial optimization for each slide. Two different subregions of the hippocampus (CA1 and DG) were imaged. Areas of analysis from the CA1 subregion were optically sectioned at approximately 0.75 µm in the z-plane. Three different CA1 regions per animal were imaged in triplicate with an average of 900 cells analyzed per rat. Cells were counted by an experimenter blind to the conditions using a software package called 3D-catFISH (Chawla et al., 2004) that allowed a quantitative analysis of temporal gene transcription activity imaged by fluorescence in situ hybridization known as the “catFISH” method (Guzowski et al., 1999). The program first segments nuclei that have been counterstained with DNA dyes such as Sytox, using a 3-dimensional watershed algorithm. Next, the fluorescence in situ hybridization (FISH) signal present in either the nucleus or cytoplasm can typically be integrated and spatially located. Here, as reported in the Results section, the “catFISH” program was used to obtain the total number of *c-fos*-positive cells.

The entire DG was imaged from 2–3 slides per animal using the 10× objective and the confocal pinhole opened wide such that all the labeled cells were visible in a single optical plane. Images were connected off-line using MetaMorph image analysis software (Version 7.1). The montaging procedure was done as follows: 2 reconstructed DG sections per rat from the middle planes of overlapping 10× Z-stacks were used for the analysis. The area of the granule cell layer and the total number of neurons was assessed in each reconstructed flat image. The area was used to estimate the total number of neurons using a correction factor that represented the total neurons per square micron. This factor was derived from 92 Z-stacks from 10 different rats collected at 40× magnification. The total number of neurons per stack was counted and the area of the granule cell layer (in µm²) from the middle plane was calculated (Chawla et al., 2005; Ramirez-Amaya et al., 2005). Using this factor, the percent of neurons with *c-fos* mRNA in the DG of each rat was calculated according to the following formula:

$$100 \times p / (A_p \times [N/A]),$$

where:

p = the number of *c-fos* (positive) neurons in a given reconstructed flat image;

A_p = the area (in μm^2) of the DG, as measured from the reconstructed flat image;

N = the total number of cells from all 40 $\times$ Z-stacks; and

A = the total area (in μm^2) of the DG from the middle planes of all 40 $\times$ Z-stacks.

c-fos mRNA-positive cells were counted using the spot-count capability within the semi-automated 3D-catFISH image analysis software (Chawla et al., 2004; Lin et al., 2005). The total number of *c-fos* cells were counted as positive if fluorescence staining was present in the nucleus, cytoplasm, or both.

2.6. RT-PCR

2.6.1. RNA extraction and cDNA synthesis—After behavioral treatments animals were decapitated with a rodent guillotine and the hippocampus was dissected out, with the CA1 and DG regions separated and flash frozen in liquid nitrogen. All brain tissue was processed in batches so that all samples from each of the time points for the behavior and seizure conditions were processed together. Hippocampal samples used for RT-PCR were disrupted in 200 μL of lysis/binding solution and total RNA samples were prepared using the RNeasy-4PCR (Ambion, Austin, TX, USA) kit according to the manufacturer's instructions. Briefly, after tissue disruption, a 64% ethanol solution was added to each sample and passed through filter cartridges to bind RNA. The filters were washed 3 times to remove residual DNA, protein, and contaminants. RNA was then eluted from the washed filters and DNase-treated to remove any remaining contaminating DNA before cDNA synthesis. Samples were stored at -70°C until further use.

Total RNA was reverse transcribed into cDNA using the Super-Script II First Strand Synthesis System for RT-PCR (Invitrogen, Eugene, OR, USA) according to the manufacturer's instructions. Briefly, RNA (2 μg) was incubated for 10 minutes at 70°C in a total reaction volume of 11 μL with oligo-dT primers (5 ng/ μL) and chilled on ice. A cDNA synthesis buffer (4 μL), 0.1 M DTT (2 μL) and 10 mM dNTPs were added to the reaction and incubated at 45°C for 2 minutes. Reverse transcriptase (1 μL ; 200 units SuperScript II) was added to the reaction and incubated at 45°C for 60 minutes. cDNA was diluted in 1X TE and stored at -20°C . A negative control was included in which no reverse transcriptase was added.

2.6.2. Primers and real-time PCR—Primers for *c-fos* and for glyceraldehyde-3-phosphate-dehydrogenase (GapDH), were designed using Primer 3 software (www.genome.wi.mit.edu) based on the rat sequences deposited in GenBank at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/ezproxy>). GapDH was used to normalize data as it is stably and constitutively expressed at high levels in most cells and tissues and its expression remains unchanged with age or various treatments (Tanic et al., 2007). The primer sequence for GapDH was as follows: 5'-AATGGGAGTTGCTGTTGAAG-3' (forward), 5'-CTGGAGAAACCTGCCAAGTA-3' (reverse). The primer sequence for *c-fos* was as follows: 5'-CCCGTAGACCTAGGGAGGAC-3' (forward), 5'-CAATACACTCCATGCGGTTG-3' (reverse). Primer specificity was verified by melt curve analysis.

PCR amplification of cDNA was performed using the BioRad iCycler Real-Time Detection System (BioRad Laboratories, Hercules, CA, USA). cDNA (4 μL) was added to a 1X reaction master mix (46 μL) containing 3 mM MgCl_2 , KCl, Tris-HCl, iTaq DNA polymerase, SYBR Green I (25 U/mL), dNTPs, fluorescein (10 nM), and gene-specific primers (approximately 500 nM each of forward and reverse primer). For each experimental sample, duplicate reactions were conducted in 96-well plates (BioRad). PCR cycling

conditions consisted of hot-start activation of iTaq DNA polymerase at 95 °C and 40 cycles of denaturation (95 °C, 30 seconds), annealing (56 °C, 30 seconds), and extension (72 °C, 30 seconds). A melt curve analysis was conducted to determine the uniformity of product formation, primer-dimer formation, and amplification of nonspecific products. PCR product was denatured (95 °C, 1 minute) before melt curve analysis, which consisted of incrementally increasing reaction temperature every 10 seconds from 58 °C to 95 °C. The negative first derivative of the melt curve (fluorescence vs. temperature) plotted against temperature will yield a single peak (T_m of product) if primers are specific to the gene of interest. All primers generated a single amplification product at a temperature greater than 77 °C.

For each RT-PCR an RT control was included, which had no RT enzyme added to the reaction mix. Each sample was run in duplicate, and GapDH was used to normalize data. The threshold for detection of PCR product above background was set at 10 times the standard deviation of the mean background fluorescence for all reactions. Background fluorescence was determined from cycles 1–5 before the exponential amplification of product and subtracted from the raw fluorescence of each reaction/cycle. The threshold for detection of a PCR product fell within the log-linear phase of amplification for each reaction. Threshold cycle (C_T ; number of cycles to reach threshold of detection) was determined for each reaction.

Because of the different types of quantitative information that can be obtained from FISH and RT-PCR methods, we have applied both of these approaches in individual rats (1 hemisphere for FISH, the other hemisphere for PCR). The goal was to identify the numbers of cells activated by the treatment as indicated by the counts of *c-fos* mRNA-positive cells (FISH), as well as the levels of *c-fos* transcripts in these specific areas of interest (PCR) within each rat.

2.7. Data analysis—For the spatial and visual discrimination trials of the Morris swim task, data were analyzed using a 2-way analysis of variance (ANOVA) with repeated measures followed by the post hoc Fisher's PLSD test ($n = 19$ old, and $n = 19$ young). The time spent in the target quadrant was analyzed using a 1-way ANOVA and a post hoc Fisher's PLSD test.

To assess group differences for cell count data, an ANOVA followed by either a Fisher's post hoc test or the Bonferroni post hoc analysis to correct for multiple comparisons was used.

Relative gene expression for RT-PCR analysis was determined using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001; Pfaffl, 2001). The mean C_T of duplicate measures was computed for each sample and the sample mean C_T of GapDH (the internal control) was subtracted from the sample mean C_T of *c-fos* (ΔC_T). The average C_T of the samples from untreated (caged control) animals for *c-fos* and GapDH were then subtracted from the mean ΔC_T of each experimental sample ($\Delta\Delta C_T$). To determine differences in the relative levels of *c-fos* mRNA between young and old animals at base-line and the following behavior, the $\Delta\Delta C_T$ was determined for each sample (C_T *c-fos* – C_T GapDH) with the young samples used as the calibrator samples in place of the caged control samples. Unpaired *t* tests were used to determine mRNA levels measured by RT-PCR. For all tests, the null hypotheses were rejected at the 0.05 level of significance.

3. Results

The Morris swim task was used to evaluate young and old animals that were assigned to rest and spatial exploration groups. Older animals were impaired in the Morris swim task as assessed by the corrected integrated path lengths over the 4-day training period (Fig. 1A). There was a statistically significant age effect ($F(1,123) = 11.636$; $p = 0.0015$) and post hoc analyses indicated a significant age difference on each of the 4 days. Additionally, younger rats spent significantly more time in the target quadrant than did older animals ($F(1,34) = 7.202$; $p = 0.01$; Fig. 1B) but there was no significant overall effect of age on the visual platform test ($F(1,40) = 2.020$; $p = 0.16$; Fig. 1C). This indicates that visual impairment in older rats was not responsible for their learning difficulties.

Although the IEGs *Arc* and *Homer 1a* have been used successfully as activity markers for the catFISH method because they distribute discretely in the nuclear and cytoplasmic cell compartments, the *c-fos* transcriptional signal (compare Fig. 2A and B) is not as distinctly localized as other effector IEGs (i.e., *Arc* and *Homer 1a*) (Guzowski et al., 1999; Vazdarjanova et al., 2002). Therefore, classification of the *c-fos* staining pattern based on the location of the FISH signal (nuclear, cytoplasmic, or both nuclear and cytoplasmic) could not be used reliably, instead we used the total number of *c-fos*-positive cells.

Under resting conditions there are few *c-fos* mRNA-positive cells in the CA1 region. The pattern of *c-fos* expression as assessed by cell counts using FISH was similar in animals from both age groups ($F(1,17) = 0.385$; $p = 0.54$; Fig. 2C). Aged rats however had significantly lower levels of *c-fos* mRNA relative to young rats ($F(1,20) = 4.998$; $p = 0.04$; Fig. 2D). Thus, *c-fos* transcription is affected by age under resting conditions and this effect is not because of changes in proportions of cells that transcribe *c-fos*. Rather it appears that pyramidal cells transcribe less *c-fos* mRNA. These data are consistent with lowered expression levels in CA1 of another IEG, *Arc* under resting conditions (Penner et al., 2011). Representative confocal images (Fig. 2E and F) illustrate the low *c-fos* expression in old and young caged control rats, respectively.

After a single 5-minute exploratory behavior session the total proportion of hippocampal CA1 neurons that transcribed *c-fos* did not differ between young and aged animals ($F(1,34) = 1.577$; $p = 0.56$; Fig. 3A). Data obtained from the 5-minute group using RT-PCR analysis, however, reveals that old animals exhibit lower *c-fos* mRNA fold change compared with caged animals and have significantly less *c-fos* mRNA relative to young rats ($F(1,9) = 8.402$; $p = 0.02$; Fig. 3B). The reduced *c-fos* activation after behavior might in part be because of lower resting *c-fos* mRNA levels in these cells. Examples of the 5-minute expression pattern of *c-fos* mRNA is shown in confocal images in Fig. 3C (old) and Fig. 3D (young).

In animals that were given two 5-minute exploration sessions separated by a 20-minute rest interval in their home cages, there was no significant difference in the proportion of *c-fos*-positive cells in the CA1 subregion with respect to age ($F(1,43) = 1.266$; $p = 0.31$; Fig. 4A). RT-PCR analysis revealed that both young and old rats show a similar fold increase in *c-fos* from resting levels ($F(1,10) = 0.114$; $p = 0.74$; Fig. 4B). However, the level transcribed per cell differed significantly, with older animals expressing a significantly lower RNA level compared with young animals ($F(1,10) = 18.683$; $p = 0.002$; Fig. 4B). Confocal images from old and young animals after the 2 behavioral sessions are shown in Fig. 4C and D, respectively.

For the granule cells in the DG, caged control rats showed no age-related changes in either the numbers of cells counted using FISH ($F(1,29) = 0.827$; $p = 0.83$; Fig. 5A) or the level of

c-fos mRNA as analyzed with RT-PCR ($F(1,19) = 0.190$; $p = 0.67$; Fig. 5B). Confocal images of old and young caged control rats are shown in Fig. 5C and D, respectively.

Animals that were given a single 5-minute behavior session exhibited no age-related changes in the numbers of *c-fos*-positive granule cells ($F(1,14) = 0.505$; $p = 0.50$; Fig. 6A). RT-PCR revealed that old and young rats had similar *c-fos* fold change compared with resting levels ($F(1,10) = 0.508$; $p = 0.49$; Fig. 6B) but the relative mRNA was reduced in old relative to young animals resulting in reduced transcript levels with age ($F(1,10) = 6.505$; $p = 0.03$; Fig. 6B). For confocal images see Fig. 6C (old) and Fig. 6D (young).

As can be seen in Figs. 7C and D, the upper blade of the DG contained significantly more *c-fos* mRNA-positive cells as compared with the lower blade, after 2 behavior sessions, similar to the IEG *Arc* (Chawla et al., 2005). With RT-PCR, there was a significant reduction in fold increase of *c-fos* mRNA in old compared with young rats with the 2 environment exposures ($F(1,10) = 14.981$; $p = 0.003$; Fig. 7B). Additionally, granule cells in young animals showed significantly higher levels of *c-fos* mRNA ($F(1,10) = 7.745$; $p = 0.02$; Fig. 7B) as compared with older rats. Thus, though the proportion of hippocampal granule cells that expressed *c-fos* in aged and young animals was not significantly different ($F(1,16) = 0.243$; $p = 0.63$; Fig. 7A), because it appeared that older rats have significantly fewer *c-fos* transcripts after behavior tasks, relative to young rats. Representative confocal images from old and young rats are shown in Fig. 7C and D, respectively.

Animals administered the seizure treatments showed dramatic *c-fos* mRNA induction in both CA1 and DG hippocampal subregions. Because transcriptional foci appear within 5 minutes of behavioral exploration or seizure induction, it was possible to count the numbers of cells expressing *c-fos* mRNA at the shorter time points; at the longer time intervals, almost all of the granule cells expressed *c-fos* (Fig. 10A and B). The proportions of cells expressing *c-fos* mRNA, 5 minutes after MECS, did not differ in young and old animals in either the CA1 and DG regions ($F(1,36) = 2.617$; $p = 0.33$; Fig. 8A; and $F(1,46) = 0.568$; $p = 0.53$; Fig. 8E). However, when we assessed the transcript levels by RT-PCR, there were significant regional differences between old and young animals. In the CA1 subregion old animals expressed significantly less fold change compared with caged control rats and had significantly less mRNA relative to young rats ($F(1,10) = 8.563$; $p = 0.02$; and $F(1,10) = 5.080$; $p = 0.049$; Fig. 8B). In DG granule cells there was equivalent *c-fos* mRNA expression regardless of age ($F(1,10) = 0.002$; $p = 0.97$; Fig. 8F), but older animals showed a significantly lower fold change in *c-fos* mRNA when compared with caged control animals ($F(1,10) = 4.356$; $p = 0.049$; Fig. 8F). Representative confocal images of old and young rats after MECS (5 minutes) are in Fig. 8C and D (CA1) and Fig. 8G and H (DG).

At the 30-minute interval after MECS treatment similar proportions of *c-fos* mRNA expressing CA1 pyramidal cells were found in old and young animals ($F(1,21) = 0.112$; $p = 0.47$; Fig. 9A), but by the 1-hour interval, fewer *c-fos*-positive cells ($F(1,21) = 4.704$; $p = 0.04$; Fig. 9E) were observed in old versus young pyramidal cells. RT-PCR analysis revealed that although old and young rats showed similar fold change compared with resting *c-fos* mRNA levels in CA1 ($F(1,6) = 0.190$; $p = 0.68$; Fig. 9B and F) there was significantly higher *c-fos* mRNA transcribed at both 30- and 60-minute time points after seizure induction ($F(1,6) = 1.252$; $p = 0.05$; Fig. 9B; and $F(1,6) = 5.03$; $p = 0.04$; Fig. 9F).

In the granule cells, however, at the 30-minute post MECS time interval older animals have significantly less *c-fos* mRNA fold change relative to caged animals and relative to young rats ($F(1,10) = 129.74$; $p = 0.003$; and $F(1,10) = 33.15$; $p = 0.005$; Fig. 10C). At 1 hour after MECS when compared with caged rats old animals exhibit a lower fold change in *c-fos* mRNA but there was a significant elevation in *c-fos* expression compared with young rats

($F(1,16) = 5.01$; $p = 0.04$; Fig. 10D), suggesting slower or delayed kinetics in *c-fos* mRNA expression in older granule cells.

4. Discussion

In the present study circuit composition and expression levels of *c-fos* were evaluated in CA1 pyramidal and DG granule cells of adult and aged animals under conditions of rest, behavioral treatments, and maximal activity. The present findings provide new insights into *c-fos* transcriptional responses with aging. The primary finding suggests consistency in the networks engaged after behavioral treatments, but reduced overall levels of *c-fos* mRNA within specific hippocampal subregions. This change in transcription could contribute to the observed changes in spatial memory in the older animals.

4.1. Levels of *c-fos* at “rest”

Under the condition in which rats were sacrificed immediately upon removal from their home cages without any intervening treatment, there was no difference between age groups in the numbers of individual CA1 pyramidal cells or DG granule cells that expressed *c-fos* mRNA. For the DG, there was also no age difference in transcript levels in young and old rats as measured by RT-PCR in the opposite hemisphere of the same animals used for cell counting. For CA1, however, *c-fos* mRNA as detected by RT-PCR was reduced in older rats, suggesting that either all or some of these cells transcribe less *c-fos* mRNA. Recently, Marrone et al. (2008) have confirmed that the IEG transcription observed under resting conditions is an indication of active information processing and not simply “background noise.” If an animal is allowed to explore its environment before a rest period, IEG transcription during rest occurs in cells that responded to the previous spatial experience. Thus, the expression observed at rest in the present study probably reflects activity in cells that was induced by recent behaviors during this relatively quiescent period (i.e., moving around in their home cages). It is also possible that *c-fos* mRNA expression might occur when the hippocampus is not actively processing information from external sources, but is engaged in states conducive to consolidation. If *c-fos* expression does occur during consolidation states, then reduced transcription in CA1 pyramidal cells in older rats could reflect defective memory stabilization in these animals.

4.2. Behavioral induction of *c-fos*

When rats were allowed to explore an arena for either one 5-minute period, or two 5-minute periods separated by a 20-minute rest interval before sacrifice, there were no differences in the numbers of cells that expressed *c-fos* mRNA (detected by FISH) between age groups in either CA1 or the DG. Both behavior induction treatments resulted in more cells stained with *c-fos* compared with the caged control condition. The 5-minute and 30-minute time points were chosen based on the kinetics of *c-fos* and other IEGs that are rapidly transcribed after spatial behavior (Guzowski et al., 1999, 2001). The lack of an age-related difference in the numbers of CA1 cells responsive to exploratory behavior is similar to the IEG *Arc*, where no age-related change in numbers of cells that express *Arc* have been detected in CA1 (Penner et al., 2011; Small et al., 2004). In the DG no age-related differences were observed in numbers of cells that expressed *c-fos* mRNA at either the 5-minute or 30-minute time point. This is similar to *Arc* for the 5-minute time point; however, there are fewer *Arc*-positive granule cells in old rats at 30 minutes (Penner et al., 2011; Small et al., 2004). When transcript levels of *c-fos* were analyzed using RT-PCR, both CA1 and DG cells had reduced *c-fos* mRNA in old rats at both the 5- and 30-minute time intervals. Though some of the changes that occur with aging in *c-fos* expression are similar to those observed for *Arc*, they are not identical. This suggests that different IEGs might have differential vulnerabilities to age-related changes.

The present results showing reduced *c-fos* transcript levels in both hippocampal subregions with advancing age, parallels other investigations that have found reduced *c-fos* responses in aged memory-deficient animals (Brightwell et al., 2004; Porte et al., 2008; Verbitsky et al., 2004). Touzani et al. (2003) reported age-related decreases in levels of *c-fos* expression in septal and hippocampal regions, that were associated with short-term but not long-term retention of the radial arm maze discrimination task. Furthermore, the kinetics of *c-fos* expression might differ in young and old animals. For example, studies of *c-fos* induction in hippocampus, after seizures (induced by intravenous pentylenetetrazole) show that older rats respond more slowly to seizure onset, and experience an overall attenuation in the levels of *c-fos* mRNA (Retchkiman et al., 1996; Schmoll et al., 2001; Wagner et al., 2000). Reduced *c-fos* expression levels in aged rats cannot be attributed to neuronal loss, because hippocampal cell numbers do not significantly decrease with age in rats (Geinisman et al., 2004; Morrison and Hof, 1997; Rapp and Gallagher, 1996; Rasmussen et al., 1996). Additionally, the present data show that though both old and young rats show an increase in *c-fos* mRNA after behavior treatments compared with resting levels in CA1, older rats have significantly lower levels of mRNA relative to younger rats. Because the same proportions of neurons are activated by exploration, it is possible that some or all of the cells that transcribe *c-fos* in old rats make less RNA. It is worth noting that wherever a difference is observed in mRNA levels by RT-PCR, that difference is not apparent in confocal images shown in the figures. The reason for this discrepancy might partly be because of the fact that the confocal images are from a single optically sectioned plane (1 μ m/20 μ m thick section) and a 1-to-1 comparison between the 2 techniques is challenging. Moreover, FISH is a semiquantitative procedure that provides great cellular resolution and RT-PCR aids in the quantification of transcript levels. In addition, unlike mRNAs for *Arc*, *BDNF*, or *CamKII* that are trafficked to neuronal dendrites, no such evidence of trafficking of *c-fos* mRNA is apparent. Thus the mRNA present in the homogenized brain regions (RT-PCR) is mainly from the neuronal cell bodies.

Though the present study, using a behavioral induction paradigm suggests that there are reduced *c-fos* transcript levels with age, a previous study (Lanahan et al., 1997) observed increased *c-fos* expression in old rats after LTP induction. Experiments using artificial stimulation to induce LTP have shown that IEGs can have different stimulus thresholds for transcription induction (Abraham et al., 1993; Worley et al., 1993). The different findings in the 2 studies might therefore simply be attributed to the induction method used: behavior versus direct electrical stimulation. Specific changes in gene expression of inducible transcription factors, mRNA encoding receptor-associated proteins, and structural proteins are known to occur after different forms of neuronal activity (i.e., LTP, behavior), that can lead to unique gene expression patterns (Abraham and Williams, 2003; Bramham, 2008; Guzowski et al., 2005; Havik et al., 2007).

4.3. *c-fos* activity after MECS

Another important finding in the present study is that age-related differences emerge after a strong stimulation procedure, i.e., MECS. At 5 and 30 minutes after seizures, no age-related differences were observed in the numbers of cells that express *c-fos* in either CA1 or DG. However, at the 60-minute interval after seizure there are significantly fewer CA1 pyramidal cells in older rats that show *c-fos* expression compared with younger rats. It is possible that at this time point some pyramidal cells fail to sustain the expression of *c-fos*. Furthermore, *c-fos* transcript levels are reduced in the CA1 subregion in older rats at 5 minutes after seizure, but by 30 minutes or 1 hour, older animals display significantly higher *c-fos* transcript levels compared with younger animals. For the granule cells, no age-related difference in *c-fos* transcript level was observed at 5 minutes after seizure, but by 30 minutes, older animals showed reduced *c-fos* transcript level compared with young. One

hour after MECS, *c-fos* transcript levels were higher in older rats than in young. Induction of *c-fos* after a single MECS episode was first reported by Cole et al. (1990). Since then only a handful of studies have examined the effect of *c-fos* (Jensen et al., 1993; Retchkiman et al., 1996; Wagner et al., 2000) and aging after seizure activity. Attenuation in expression of Fos protein in various brain regions, including the hippocampus, was observed in aging mice with approximately 58%–60% reduction in Fos immunopositive cells 1 hour after electroconvulsive shock (D'Costa et al., 1991). The different findings between D'Costa et al. and the present study might be attributable to variations in either species used or translational versus transcriptional readout. One of the few studies that have compared *c-fos* mRNA used chemicals to produce seizures in young and old rats and reported a shift in the maximum *c-fos* expression from 1 hour in the young rats (3 months old) to 3 hours in old animals (28 months old), with older rats expressing only 73% of the levels expressed by young animals (Schmoll et al., 2001). Although this study appears to be inconsistent with our findings, 1 explanation for the different results might be delayed seizure onset in old rats, because of a decreased sensitivity to chemical-induced seizures in old rats (Nokubo et al., 1986; Schmoll et al., 2001).

In the central nervous system, basal expression of IEGs coding for inducible transcriptional factors is generally low, but with activation of second messenger systems these factors undergo a rapid and transient induction (Dragunow and Robertson, 1987; Morgan et al., 1987). The molecular effects of induced seizures are diverse, including increases in levels of neurotrophic factors, neurotransmitters, neuropeptides, and increases in synaptic remodeling (Fochtmann, 1994). Epidemiologic studies have shown that aging is associated with an increased incidence of seizure disorders (Hesdorffer et al., 1998; Rowan, 2005). These clinical observations are supported and extended by data from aged rodents that exhibit enhanced hippocampal-dependent susceptibility for seizures. For example, older rats show longer duration after-discharges and faster propagation to the contralateral hemisphere during kindling (Chiba et al., 1992). However, older rats show a slower kindling rate (Chiba et al., 1992; de Toledo-Morrell et al., 1984) that is mediated via NMDA (N-methyl-D-aspartate) receptors. Because decreases in NMDA-receptor mediated properties also occur with age (Barnes et al., 2000; Magnusson, 2000; Wenk and Barnes, 2000), the slower kindling rate is likely because of diminished age-related plasticity rather than increased seizure susceptibility, per se.

In some respects, the pattern of *c-fos* expression in the aged rat hippocampus is well preserved. For example, exploratory behavior engages the same numbers of cells that express *c-fos* mRNA. What tends to differ between age groups is the transcript level within these cells, and this varies depending upon the intensity of stimulus used. After behavior activity, old animals generally show dysregulation that manifests itself as lower *c-fos* mRNA expression, which might have negative consequences for overall transcription in hippocampal cells. On the other hand, seizures (MECS) in older rats result in *c-fos* overexpression, which could potentially lead to increased excitotoxicity in these cells. These age-related changes in IEG expression pattern, either too low or too high, might result in ineffective plastic responses leading to cognitive decline on the one hand, or increased vulnerability to damage or disease on the other. Disruption of these plasticity mechanisms might lead to cognitive deficits experienced by aged animals. Thus, the novel combination of methods applied to the question of how behavior dynamically regulates activity in circuits critical for memory provides a conceptual framework for understanding the neural basis of memory decline. It would be interesting to determine whether strategies could be developed to achieve a more balanced or regulated state in these aging cells, and whether such treatments would benefit cognition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Abraham WC, Dragunow M, Tate WP. The role of immediate early genes in the stabilization of long-term potentiation. *Mol. Neurobiol.* 1991; 5:297–314. [PubMed: 1688055]
- Abraham WC, Mason SE, Demmer J, Williams JM, Richardson CL, Tate WP, Lawlor PA, Dragunow M. Correlations between immediate early gene induction and the persistence of long-term potentiation. *Neuroscience.* 1993; 56:717–727. [PubMed: 8255430]
- Abraham WC, Williams JM. Properties and mechanisms of LTP maintenance. *Neuroscientist.* 2003; 9:463–474. [PubMed: 14678579]
- Barnes CA, Rao G, Houston FP. LTP induction threshold change in old rats at the perforant path–granule cell synapse. *Neurobiol. Aging.* 2000; 21:613–620. [PubMed: 11016529]
- Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, Foster TC, Landfield PW. Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. *J. Neurosci.* 2003; 23:3807–3819. [PubMed: 12736351]
- Bramham CR. Local protein synthesis, actin dynamics, and LTP consolidation. *Curr. Opin. Neurobiol.* 2008; 18:524–531. [PubMed: 18834940]
- Brightwell JJ, Gallagher M, Colombo PJ. Hippocampal CREB1 but not CREB2 is decreased in aged rats with spatial memory impairments. *Neurobiol. Learn. Mem.* 2004; 81:19–26. [PubMed: 14670355]
- Bucci DJ, Rosen DL, Gallagher M. Effects of age on pilocarpine-induced *c-fos* expression in rat hippocampus and cortex. *Neurobiol. Aging.* 1998; 19:227–232. [PubMed: 9661997]
- Burke SN, Barnes CA. Neural plasticity in the ageing brain. *Nat. Rev. Neurosci.* 2006; 7:30–40. [PubMed: 16371948]
- Chawla MK, Guzowski JF, Ramirez-Amaya V, Lipa P, Hoffman KL, Marriott LK, Worley PF, McNaughton BL, Barnes CA. Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. *Hippocampus.* 2005; 15:579–586. [PubMed: 15920719]
- Chawla MK, Lin G, Olson K, Vazdarjanova A, Burke SN, McNaughton BL, Worley PF, Guzowski JF, Roysam B, Barnes CA. 3D-catFISH: a system for automated quantitative three-dimensional compartmental analysis of temporal gene transcription activity imaged by fluorescence in situ hybridization. *J. Neurosci. Methods.* 2004; 139:13–24. [PubMed: 15351517]
- Chiba S, Muneoka Y, Sato Y, Miyagishi T. Seizure susceptibility in aged rats–pentylenetetrazol-induced seizures and amygdaloid kindling seizures [in Japanese]. *No To Shinkei.* 1992; 44:559–564. [PubMed: 1389563]
- Clayton DF. The genomic action potential. *Neurobiol. Learn. Mem.* 2000; 74:185–216. [PubMed: 11031127]
- Cole AJ, Abu-Shakra S, Saffen DW, Baraban JM, Worley PW. Rapid rise in transcription factor messenger RNAs in rat brain after electroshock induced seizures. *J. Neurochem.* 1990; 55:1920–1927. [PubMed: 2230801]
- Cole AJ, Saffen DW, Baraban JM, Worley PF. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature.* 1989; 340:474–476. [PubMed: 2547165]
- Curran T, Morgan JI. Memories of fos. *Bioessays.* 1987; 7:255–258. [PubMed: 3124834]

- D'Costa A, Breese CR, Boyd RL, Booze RM, Sonntag WE. Attenuation of Fos-like immunoreactivity induced by a single electroconvulsive shock in brains of aging mice. *Brain Res.* 1991; 567:204–211. [PubMed: 1687805]
- Desjardins S, Mayo W, Vallee M, Hancock D, Le Moal M, Simon H, Abrous DN. Effect of aging on the basal expression of c-Fos, c-Jun, and Egr-1 proteins in the hippocampus. *Neurobiol. Aging.* 1997; 18:37–44. [PubMed: 8983031]
- de Toledo-Morrell L, Morrell F, Fleming S. Age-dependent deficits in spatial memory are related to impaired hippocampal kindling. *Behav. Neurosci.* 1984; 98:902–907. [PubMed: 6091704]
- Dragunow M, Abraham WC, Goulding M, Mason SE, Robertson HA, Faull RL. Long-term potentiation and the induction of c-fos mRNA and proteins in the dentate gyrus of unanesthetized rats. *Neurosci. Lett.* 1989; 101:274–280. [PubMed: 2505198]
- Dragunow M, Robertson HA. Generalized seizures induce c-fos protein(s) in mammalian neurons. *Neurosci. Lett.* 1987; 82:157–161. [PubMed: 2447534]
- Fochtman LJ. Animal studies of electroconvulsive therapy: foundations for future research. *Psychopharmacol. Bull.* 1994; 30:321–444. [PubMed: 7878180]
- Geinisman Y, Ganeshina O, Yoshida R, Berry RW, Disterhoft JF, Gallagher M. Aging, spatial learning, and total synapse number in the rat CA1 stratum radiatum. *Neurobiol. Aging.* 2004; 25:407–416. [PubMed: 15123345]
- Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McGaugh JL, Worley PF, Barnes CA. Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J. Neurosci.* 2000; 20:3993–4001. [PubMed: 10818134]
- Guzowski JF, McNaughton BL, Barnes CA, Worley PF. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nat. Neurosci.* 1999; 2:1120–1124. [PubMed: 10570490]
- Guzowski JF, McNaughton BL, Barnes CA, Worley PF. Imaging neural activity with temporal and cellular resolution using FISH. *Curr. Opin. Neurobiol.* 2001; 11:579–584. [PubMed: 11595491]
- Guzowski JF, Timlin JA, Roysam B, McNaughton BL, Worley PF, Barnes CA. Mapping behaviorally relevant neural circuits with immediate-early gene expression. *Curr. Opin. Neurobiol.* 2005; 15:599–606. [PubMed: 16150584]
- Havik B, Rokke H, Dagyte G, Stavrum AK, Bramham CR, Steen VM. Synaptic activity-induced global gene expression patterns in the dentate gyrus of adult behaving rats: induction of immunity-linked genes. *Neuroscience.* 2007; 148:925–936. [PubMed: 17764852]
- Herdegen T, Leah JD. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Res. Brain Res. Rev.* 1998; 28:370–490. [PubMed: 9858769]
- Hesdorffer DC, Logroscino G, Cascino G, Annegers JF, Hauser WA. Incidence of status epilepticus in Rochester, Minnesota, 1965–1984. *Neurology.* 1998; 50:735–741. [PubMed: 9521266]
- Jensen FE, Firkusny IR, Mower GD. Differences in c-fos immunoreactivity due to age and mode of seizure induction. *Brain Res. Mol. Brain Res.* 1993; 17:185–193. [PubMed: 8510493]
- Jiang H-K, Owyang V, Hong J-S, Gallagher M. Elevated dynorphin in the hippocampal formation of aged rats: relation to cognitive impairment on a spatial learning task. *Proc. Natl. Acad. Sci. U. S. A.* 1989; 86:2948–2951. [PubMed: 2565040]
- Jones MW, Errington ML, French PJ, Fine A, Bliss TV, Garel S, Charnay P, Bozon B, Laroche S, Davis S. A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nat. Neurosci.* 2001; 4:289–296. [PubMed: 11224546]
- Kaczmarek L, Nikolajew E. c-fos protooncogene expression and neuronal plasticity. *Acta Neurobiol. Exp. (Wars.).* 1990; 50:173–179. [PubMed: 2130639]
- Lanahan A, Lyford G, Stevenson GS, Worley PF, Barnes CA. Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. *J. Neurosci.* 1997; 17:2876–2885. [PubMed: 9092609]
- Lanahan A, Worley P. Immediate-early genes and synaptic function. *Neurobiol. Learn. Mem.* 1998; 70:37–43. [PubMed: 9753585]

- Lin G, Chawla MK, Olson K, Guzowski JF, Barnes CA, Roysam B. Hierarchical, model-based merging of multiple fragments for improved three-dimensional segmentation of nuclei. *Cytometry A*. 2005; 63:20–33. [PubMed: 15584021]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
- Loeblich S, Nedivi E. The function of activity-regulated genes in the nervous system. *Physiol. Rev.* 2009; 89:1079–1103. [PubMed: 19789377]
- Magnusson KR. Declines in mRNA expression of different subunits may account for differential effects of aging on agonist and antagonist binding to the NMDA receptor. *J. Neurosci.* 2000; 20:1666–1674. [PubMed: 10684868]
- Marrone DF, Schaner MJ, McNaughton BL, Worley PF, Barnes CA. Immediate-early gene expression at rest recapitulates recent experience. *J. Neurosci.* 2008; 28:1030–1033. [PubMed: 18234881]
- Morgan JI, Cohen DR, Hempstead JL, Curran T. Mapping patterns of c-fos expression in the central nervous system after seizure. *Science*. 1987; 237:192–197. [PubMed: 3037702]
- Morrison JH, Hof PR. Life and death of neurons in the aging brain. *Science*. 1997; 278:412–419. [PubMed: 9334292]
- Nokubo M, Kitani K, Ohta M, Kanai S, Sato Y, Masuda Y. Age-dependent increase in the threshold for pentylentetrazole induced maximal seizure in mice. *Life Sci.* 1986; 38:1999–2007. [PubMed: 3713434]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press Inc.; San Diego: 1986.
- Penner MR, Roth TL, Chawla MK, Hoang LT, Roth ED, Lubin FD, Sweatt JD, Worley PF, Barnes CA. Age-related changes in Arc transcription and DNA methylation within the hippocampus. *Neurobiol. Aging*. 2011; 32:2198–2210. [PubMed: 20189687]
- Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 2001; 29:e45. [PubMed: 11328886]
- Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C, Mao X, Engelsberg A, Mahlke C, Welzl H, Kobalz U, Stawrakakis A, Fernandez E, Waltereit R, Bick-Sander A, Therstappen E, Cooke SF, Blanquet V, Wurst W, Salmen B, Bosl MR, Lipp HP, Grant SG, Bliss TV, Wolfer DP, Kuhl D. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron*. 2006; 52:437–444. [PubMed: 17088210]
- Porte Y, Buhot MC, Mons N. Alteration of CREB phosphorylation and spatial memory deficits in aged 129T2/Sv mice. *Neurobiol. Aging*. 2008; 29:1533–1546. [PubMed: 17478013]
- Qian Z, Gilbert ME, Colicos MA, Kandel ER, Kuhl D. Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. *Nature*. 1993; 361:453–457. [PubMed: 8429885]
- Qiang M, Xie J, Wang H, Qiao J. Effect of nitric oxide synthesis inhibition on c-Fos expression in hippocampus and cerebral cortex following two forms of learning in rats: an immunohistochemistry study. *Behav. Pharmacol.* 1999; 10:215–222. [PubMed: 10780834]
- Ramirez-Amaya V, Vazdarjanova A, Mikhael D, Rosi S, Worley PF, Barnes CA. Spatial exploration-induced Arc mRNA and protein expression: evidence for selective, network-specific reactivation. *J. Neurosci.* 2005; 25:1761–1768. [PubMed: 15716412]
- Rapp PR, Gallagher M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93:9926–9930. [PubMed: 8790433]
- Rasmussen T, Schliemann T, Sorensen JC, Zimmer J, West MJ. Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. *Neurobiol. Aging*. 1996; 17:143–147. [PubMed: 8786797]
- Retchkiman I, Fischer B, Platt D, Wagner AP. Seizure induced C-Fos mRNA in the rat brain: comparison between young and aging animals. *Neurobiol. Aging*. 1996; 17:41–44. [PubMed: 8786801]
- Rowan AJ. Epilepsy in older adults. Common morbidities influence development, treatment strategies, and expected outcomes. *Geriatrics*. 2005; 60:30–32. [PubMed: 16343034]
- Rowe WB, Blalock EM, Chen KC, Kadish I, Wang D, Barrett JE, Thibault O, Porter NM, Rose GM, Landfield PW. Hippocampal expression analyses reveal selective association of immediate-early,

- neuroenergetic, and myelinogenic pathways with cognitive impairment in aged rats. *J. Neurosci.* 2007; 27:3098–3110. [PubMed: 17376971]
- Santin LJ, Aguirre JA, Rubio S, Begega A, Miranda R, Arias JL. c-Fos expression in supramammillary and medial mammillary nuclei following spatial reference and working memory tasks. *Physiol. Behav.* 2003; 78:733–739. [PubMed: 12782230]
- Schmoll H, Badan I, Fischer B, Wagner AP. Dynamics of gene expression for immediate early- and late genes after seizure activity in aged rats. *Arch. Gerontol. Geriatr.* 2001; 32:199–218. [PubMed: 11395167]
- Schmoll H, Ramboiu S, Platt D, Herndon JG, Kessler C, Popa-Wagner A. Age influences the expression of GAP-43 in the rat hippocampus following seizure. *Gerontology.* 2005; 51:215–224. [PubMed: 15980649]
- Shen J, Barnes CA. Age-related decrease in cholinergic synaptic transmission in three hippocampal subfields. *Neurobiol. Aging.* 1996; 17:439–451. [PubMed: 8725906]
- Silva AJ, Giese KP. Plastic genes are in! *Curr. Opin. Neurobiol.* 1994; 4:413–420.
- Small SA, Chawla MK, Buonocore M, Rapp PR, Barnes CA. Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proc. Natl. Acad. Sci. U. S. A.* 2004; 101:7181–7186. [PubMed: 15118105]
- Smith DR, Hoyt EC, Gallagher M, Schwabe RF, Lund PK. Effect of age and cognitive status on basal level AP-1 activity in rat hippocampus. *Neurobiol. Aging.* 2001; 22:773–786. [PubMed: 11705637]
- Tanic N, Perovic M, Mladenovic A, Ruzdijic S, Kanazir S. Effects of aging, dietary restriction and glucocorticoid treatment on housekeeping gene expression in rat cortex and hippocampus-evaluation by real time RT-PCR. *J. Mol. Neurosci.* 2007; 32:38–46. [PubMed: 17873286]
- Teather LA, Packard MG, Smith DE, Ellis-Behnke RG, Bazan NG. Differential induction of c-Jun and Fos-like proteins in rat hippocampus and dorsal striatum after training in two water maze tasks. *Neurobiol. Learn. Mem.* 2005; 84:75–84. [PubMed: 15936959]
- Tischmeyer W, Grimm R. Activation of immediate early genes and memory formation. *Cell. Mol. Life Sci.* 1999; 55:564–574. [PubMed: 10357227]
- Touzani K, Marighetto A, Jaffard R. Fos imaging reveals ageing-related changes in hippocampal response to radial maze discrimination testing in mice. *Eur. J. Neurosci.* 2003; 17:628–640. [PubMed: 12581181]
- Vazdarjanova A, McNaughton BL, Barnes CA, Worley PF, Guzowski JF. Experience-dependent coincident expression of the effector immediate-early genes arc and Homer 1a in hippocampal and neocortical neuronal networks. *J. Neurosci.* 2002; 22:10067–10071. [PubMed: 12451105]
- Verbitsky M, Yonan AL, Malleret G, Kandel ER, Gilliam TC, Pavlidis P. Altered hippocampal transcript profile accompanies an age-related spatial memory deficit in mice. *Learn. Mem.* 2004; 11:253–260. [PubMed: 15169854]
- Wagner AP, Schmoll H, Badan I, Platt D, Kessler C. Brain plasticity: to what extent do aged animals retain the capacity to coordinate gene activity in response to acute challenges. *Exp. Gerontol.* 2000; 35:1211–1227. [PubMed: 11113603]
- Wenk GL, Barnes CA. Regional changes in the hippocampal density of AMPA and NMDA receptors across the lifespan of the rat. *Brain Res.* 2000; 885:1–5. [PubMed: 11121523]
- Worley PF, Bhat RV, Baraban JM, Erickson CA, McNaughton BL, Barnes CA. Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement. *J. Neurosci.* 1993; 13:4776–4786. [PubMed: 8229198]

Fig. 1.

There is a significant age-related impairment in the acquisition of the spatial version of the Morris swim task. (A) Older rats exhibited longer corrected integrated path lengths (CIPL) on all 4 training days when compared with the younger rats (* $p < 0.05$). (B) During the probe trial older animals spent significantly less time in the target quadrant (* $p = 0.01$) than did the younger rats. (C) On the cued (visible platform) portion of the swim task older and young rats showed improved performance from day 1 to day 2 but there was no significant effect of age on the path lengths ($p > 0.05$).

Fig. 2.

Proportions of CA1 pyramidal cells that express *c-fos* mRNA by fluorescence in situ hybridization (FISH) and levels of *c-fos* determined by polymerase chain reaction (PCR) in animals given no behavior treatment. (A and B) Confocal images show differences between the transcription signal of *c-fos* versus another immediate-early gene (IEG) *Arc*. Notice the discrete distribution of *Arc* in the nuclear and cytoplasmic compartments whereas *c-fos* shows a more diffuse distribution. (C) Similar numbers of pyramidal cells express *c-fos* mRNA in young and old rats under a caged-control or “resting” condition. (D) Resting *c-fos* mRNA (caged controls) as determined by reverse transcription (RT)-PCR is significantly lower in old animals relative to young animals (star, $p < 0.0001$). Confocal images from the hippocampal CA1 region of an old (E) and young (F) rat (magnification $\times 250$). Very few cells express *c-fos* mRNA in the no behavior treatment condition in either group. Nuclei are counterstained with Sytox (blue) and *c-fos* mRNA expression is indicated in red (Cy3).

Fig. 3.

Proportions of CA1 pyramidal cells that express *c-fos* mRNA by fluorescence in situ hybridization (FISH) and levels of *c-fos* mRNA by reverse transcription polymerase chain reaction (RT-PCR) in animals that were given a single 5-minute exploration session (5' A). (A) The proportions of pyramidal cells (total) that express *c-fos* mRNA are similar in old and young animals after the 5-minute behavioral exploration treatment. (B) RT-PCR analysis reveals that old animals show a significantly reduced fold increase in *c-fos* mRNA compared with resting levels and *c-fos* mRNA levels which are significantly (*=significant) lower in old compared with young rats (star, $p < 0.05$). (C) CA1 pyramidal cell region illustrating *c-fos* staining after fluorescence in situ hybridization (confocal image taken at magnification $\times 250$) taken from an old animal. The nuclear counterstain is Sytox (shown in blue), and the *c-fos* positive staining (red) can be seen within the nuclear compartment. (D) Similar confocal image obtained from a young rat.

Fig. 4.

Proportions of CA1 pyramidal cells that express *c-fos* mRNA by fluorescence in situ hybridization (FISH) and levels of *c-fos* mRNA by reverse transcription polymerase chain reaction (RT-PCR) from animals that explored a defined environment twice for 5 minutes separated by a 20-minute rest interval (A-20'-A). (A) The proportions of *c-fos* positive cells activated by this behavior treatment are not significantly different between the 2 age groups. The total proportions reflect cells with nuclear staining (foci), cytoplasmic, or both nuclear and cytoplasmic staining. (B) *c-fos* mRNA measured with RT-PCR indicates that both old and young animals show a similar fold increase from resting levels and that the relative mRNA levels of *c-fos* in old animals is significantly lower compared with young animals (star, $p < 0.05$). (C and D) Confocal images from an old and young rat respectively, illustrate pyramidal cell nuclei (counterstained with Sytox, blue) and *c-fos* mRNA staining (Cy3; red).

Fig. 5.

The proportions of granule cells in young and old rats that express *c-fos* mRNA by fluorescence in situ hybridization (FISH) and levels of *c-fos* reverse transcription polymerase chain reaction (RT-PCR) after no behavior treatment (DG no behavior). (A) Similar proportions of granule cells express *c-fos* mRNA in young and old rats under a caged-control or “resting” condition. (B) No age-related differences in *c-fos* mRNA as determined by RT-PCR were detected in this behavior control condition. Representative confocal images from the dentrate gyrus of old (C) and young (D) rats. Nuclei are counterstained with sytox (blue) and *c-fos* mRNA expression in red (Cy3).

Fig. 6.

The proportions of granule cells in young and old rats that express *c-fos* mRNA by fluorescence in situ hybridization (FISH) and levels of *c-fos* reverse transcription polymerase chain reaction (RT-PCR) after a single 5-minute exploratory behavior treatment (DG 5'-A). (A) No significant age differences were detected in the proportions of granule cells that express *c-fos* by FISH. (B) Although both age groups showed similar fold change in *c-fos* mRNA from resting levels, there is a significant difference by RT-PCR in animals that received the single 5-minute behavioral treatment with old rats having lower *c-fos* mRNA levels relative to young rats. Representative confocal images from the dentate gyrus of old (C) and young (D) rats.

Fig. 7.

The proportions of granule cells using fluorescence in situ hybridization (FISH) and levels of *c-fos* mRNA obtained with reverse transcription polymerase chain reaction (RT-PCR) in animals that explored a defined environment for 5 minutes followed by a rest period in the home cage for 20 minutes and finally were given another 5 minutes exploration session in the same environment (DG A-20'-A). (A) Proportions of *c-fos* positive cells in the dentate gyrus are similar in young and old animals after 2 exploration periods (star, $p > 0.05$). (B) Old rats exhibit a significantly reduced fold change in *c-fos* mRNA level compared with resting levels by RT-PCR and have lower *c-fos* transcript levels compared with young rats (star, $p < 0.05$). (C and D) Confocal images of the dentate gyrus (DG) from an old and young rat. Note most of the staining is present in the upper blade (top blade in figure) of DG. Nuclei are shown in blue and *c-fos* staining in red (Cy3).

Fig. 8.

Fluorescence in situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR) data from animals that were given maximal electroconvulsive shock (MECS) treatment and sacrificed after 5 minutes (CA1 5' MECS). (A) In area CA1 there was no difference in proportions of *c-fos*-positive cells between age groups. (B) Results from the RT-PCR analysis reveals an age-related decrease (*=significant) in *c-fos* fold change in older animals (star, $p < 0.05$) when compared with resting levels and also a decrease in *c-fos* transcript level relative to young animals. (E) In the dentate gyrus, there is no age-related difference in the numbers of granule cells that express *c-fos* but old rats show a significant decrease in fold change from resting levels (F) as analyzed with RT-PCR. In addition, old animals have similar relative *c-fos* mRNA levels compared with young animals because no significant difference in *c-fos* transcript level was detected by RT-PCR (F). (C and D) Confocal images taken from the CA1 region of an old and young rat respectively; (G and H) are confocal images from the upper blade of an old and young rat, respectively.

Fig. 9.

Fluorescence in situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR) data from area CA1 of old and young rats that were given maximal electroconvulsive shock (MECS) and sacrificed either 30 minutes (CA130' MECS) or 60 minutes (CA160' MECS) later. FISH reveals that the proportions of cells containing *c-fos* mRNA are not significantly different between young and old animals at the 30-minute time point (A) but at the 60-minute time point (E) significantly fewer CA1 cells express *c-fos* mRNA in the old group. RT-PCR indicates that old animals exhibit a similar fold change in *c-fos* mRNA when compared with resting levels but have a significantly lower relative *c-fos* mRNA transcript level when compared with young animals at both the 30- and 60-minute intervals (B and F). Panels C (30 minutes) and G (60 minutes) are confocal images from old MECS-treated rats and panels D (30 minutes) and H (60 minutes) are examples from young rats. CA1 pyramidal cells are counterstained with Sytox (blue) and *c-fos* mRNA is shown in red (Cy3).

Fig. 10.

Representative confocal images from the dentate gyrus (DG) 30 minutes after maximal electroconvulsive shock (MECS) (DG 30' MECS), 60 minutes after MECS (DG 30' post MECS) images were similar to images shown. (A) Image from an old rat and (B) is from a young animal. *c-fos* mRNA is shown in red (Cy3) and nuclei are counterstained with Sytox (blue). (C) A lower fold change in old animals compared with resting levels and lower *c-fos* mRNA transcript levels in old animals relative to young. (D) indicates that older animals at the 1-hour post MECS (60' post MECS) treatment also have a lower fold change in *c-fos* mRNA level but express significantly higher *c-fos* mRNA transcript levels relative to young animals (star, $p > 0.05$).