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Age-changes in gene expression in primary mixed glia cultures from young vs old rat cerebral cortex are modified by interactions with neurons

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Abstract

Astrocytic GFAP expression increases during normal aging in many brain regions and in primary astrocyte cultures derived from aging rodent brains. As shown below, we unexpectedly found that the age-related increase of GFAP expression was suppressed in mixed glia (astrocytes + microglia). However, the age-related increase of GFAP was observed when E18 neurons were co-cultured with mixed glia. Thus, the presence of microglia can suppress the age-related increase of GFAP, in primary cultures of astrocytes. To more broadly characterize how aging and co-culture with neurons alters glial gene expression, we profiled gene expression in mixed glia from young (3 mo) and old (24 mo) male rat cerebral cortex by Affymetrix microarray (Rat230 2.0). The majority of age changes were independent of the presence of neurons. Overall, the expression of 2-fold more genes increased with age than decreased with age. The minority of age changes that were either suppressed or revealed by the presence of neurons may be useful to analyze glial-neuron interaction during aging. Some in vitro changes are shared with those of aging rat hippocampus in studies from the Landfield group (Rowe et al., 2007; Kadish et al., 2009).

Keywords

GFAP; mixed glia; aging; Affymetrix microarray; glialneuron interactions

1. Introduction

Declines of human cognitive processing (crystalized and fluid intelligence) and processing speed begin by age 30 as determined from large community-based samples (McArdle et al., 2002; Finkel et al., 2007). We have argued that inflammatory processes of normal aging with activation of astrocytes and microglia underlie these functional changes (Finch, 2009, 2010; Finch and Morgan, 2007).

Before midlife, humans and animal models show increased astrocyte cell volume and levels of GFAP (Hansen et al., 1987; Morgan et al., 1999), the intermediate filament of astrocytes, and increased inflammatory gene expression in astrocytes and microglia (Finch, 2009; Lucin

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and Wyss-Corey, 2009; Middeldorp and Hol, 2011; Salminen et al., 2011; Sparkman and Johnson, 2009; von Bernhardi et al., 2010). Glial changes progress into later ages in both grey and white matter in many brain regions. The increased levels per cell of GFAP are due to increased rates of GFAP transcription, as shown by in situ hybridization with intron-cRNA probe (Morgan et al., 1999).

Concurrently with glial activation, synaptic density declines in some brain regions, but without clinical-grade neurodegeneration or neuron loss. For example, in the cerebral cortex of healthy human brains aged 25–99 years, astrocyte activation increased progressively (Hansen et al., 1987). These same brains also showed a decrease of synaptic density (immune-reactive PSD-95 terminals) and a shift to smaller sized neuronal perikarya (Masliah et al., 1993). These reciprocal changes are definitive by age 60 and arise without changes in the total neuron density in brains that had no indication of Alzheimer disease or other neurodegenerative conditions.

We have hypothesized that glial activation during normal aging contributes to age-related synapse loss as part of a general process of neuronal atrophy that begins soon after maturation (Rozovsky et al., 2005; Finch, 2009). This hypothesis challenges the assumption from classical neuropathology that glial activation during normal aging is secondary to neurodegeneration. These processes begin soon after maturation in healthy humans and in long-lived rodents. Nonetheless, neuronal impairments at any age can cause activation of glia, e.g. rapid astrocyte GFAP induction results from inhibition of axonal electrical activity by tetrodotoxin (Canady et al., 1994). Thus, we may consider that aging involves a complex set of reciprocal interactions between neurons and glia, which may begin during later stages of maturation.

To test hypotheses about the role of aging glia in neuronal atrophy, we have developed in vitro systems of glia cultured from adult rodent brains of different ages (heterochronic culture models). These models are based on the persistence of in vivo glial activation during primary cultures: enriched astrocyte cultures (<5% microglia and oligodendroglia) maintain the in vivo aging phenotype of elevated GFAP mRNA and protein per cell (astrocyte marker) (Rozovsky et al., 1998), while mixed glia (3:1, astrocytes:microglia) maintain elevations of IL-6 and other cytokines, and of CD-68, a monocytic-lineage marker (Xie et al., 2003; Wong et al., 2005). By manipulating GFAP expression in enriched astrocytes of different ages in co-culture with E18 cortical neurons, we showed that neurite outgrowth varied inversely with astrocyte GFAP per cell (Rozovsky et al., 2005). Similarly, mixed glia from aging rat cerebral cortex impaired neurotrophic support of E18 neurite outgrowth (Arimoto et al., in prep.).

However, as shown below, we unexpectedly found that the age-related increase of GFAP expression in mixed glia depends on the presence of E18 neurons. Thus, microglia can suppress the age-related increase of GFAP, at least in conventional primary culture. To more broadly characterize how aging and co-culture with neurons alters glial gene expression, we analyzed gene expression by Affymetrix microarrays in mixed glia from young and old rats, co-cultured with or without neurons. Analysis of this new model reveals a substantial group of glial gene changes during aging besides GFAP that are influenced by co-culture with neurons. This preliminary analysis considers general functional categories of genes (GO and KEGG), but does not address networks or pathways.

Lastly, we compare our findings on glial gene expression in vitro with those of the aging rat hippocampus reported by Phillip Landfield and colleagues (Rowe et al., 2007; Kadish et al., 2009). These studies are exemplary for rigorous analysis of microarray profiles of intermediate postmaturation ages up through the lifespan and correlated with cognitive age

changes. Age changes in glial gene expression in vitro grown with neurons may help resolve in vivo roles of aging glia in neurodegenerative processes of aging.

2. Materials and methods

2.1. Glial cultures

Primary glia were cultured from adult male rat cerebral cortex by standard procedures for mixed glia (Rozovsky et al., 2005; Xie et al., 2002). The mixed glia contained 3:1, astrocytes:microglia. Astrocytes were freed of microglia in mixed glial cultures by shaking (>95% astrocytes, <5% microglia and oligodendroglia) ('enriched astrocytes'). Glia were grown in DMEM/F12 media (Cellgro, Herndon, VA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 100 U/ml penicillin, 50 U/ml streptomycin, and 2 mM l-glutamine at 37 C, 5% CO₂. After replating, half of the cultures were seeded with embryonic (E18) cortical neurons (Rozovsky et al., 2005) and maintained as co-cultures for 2 days before harvest.

2.2. In situ hybridization

Cell content of GFAP mRNA was assayed by in situ hybridization with a cRNA probe (full length coding sequence, 2.7kb) (Laping et al., 1994). Grains per cell were measured using computer-assisted image analysis (IPlab Spectrum software, Signal Analytics Corp) on emulsion-dipped slides (Morgan et al., 1999): 250 cells on 3 separate slides per condition were counted. Average GFAP grain density in young was similar in enriched astrocytes (113±30) and mixed glia (110±15). In contrast, in old-derived cultures, grain density in enriched astrocytes (340±80) exceeded that in mixed glia (70±15).

2.3. Microarray assay and data analysis

RNA levels were profiled from mixed glia from young adult (3 mo) and old (24 mo) male rat cerebral cortex, grown with/without neurons in a 2×2 block design: Young and Old mixed glia + E18 neurons (YM, OM, YMN, OMN). Triplicate cultures were grown as above and extracted for total RNA by TRIzol reagent (GIBCO BRL, Gaithersburg MD)(Xia et al., 2002). RNA integrity was assessed electrophoretically (2100 Bioanalyzer, Agilent), with a proprietary algorithm for the ratios of 4S, 18S, and 28S species (RNA Integrity Number > 9). Total RNA was hybridized with 0.05 mg/ml for 16 hours on the Affymetrix Rat230 2.0 chip.

Microarray data were normalized using pairwise, sub-array normalization. In brief, this procedure normalized each target microarray against a reference as follows. Each Rat230 2.0 chip, consisting of 834 by 834 probes, was decomposed into sub-arrays of 50 by 50 spatially adjacent blocks. For each corresponding pair of sub-arrays on the reference and target chip, a linear relationship was estimated using least trimmed squares, and used to transform the target sub-array by the estimated shift. Adjacent sub-array overlapping of 25 by 25 probes was used to facilitate smoothing of spatial variation within each chip.

Pairwise normalizations were performed between microarrays of the different treatments. For example, in the normalization of OM data, in which one array was excluded for technical reasons, the remaining two OM microarrays (OM_i, i = 1, 2;) were each normalized against the 3 YM microarrays (YM_j, j = 1, 2, 3), resulting in 2³=6 expression values for OM (for each probeset) which were then summarized using a 3-factor probe-treatment-reference model (Cheng and Li, 2005; Li and Cheng, 2008). Data were summarized into log₂ intensity scores either for each treatment (e.g. OM) or for each microarray, as specified below. Note that normalization was performed across identical cell types.

After normalization and summarization of the data, Significance Analysis of Microarrays (SAM) (Tusher et al., 2001) was used to determine the significance of differential expression of individual probesets, using the functions from the R package “samr”; the default inputs were used in most cases, except that the number of permutations was set to 200 for the function “samr”. For input into SAM, each probeset was summarized into an individual log₂ intensity score for each microarray. The average of the log₂ intensity values for each treatment (e.g. OM) was then used as the final summarized expression value, used to determine fold change for probeset.

The primary output of the SAM analysis is a q-value (Storey, 2002) for differential expression, which serves a similar role as a P-value, but also controls for the False Discovery Rate (FDR). For our purposes, the q-value for a probeset is defined as the minimum FDR at which that probeset may be considered significant. We used two sets of cutoff criteria for individual genes in this paper: a ‘relaxed criterion’ of $q < 0.05$ and $|\log_2 \text{FC}| \log_2 1.2$ (approx. FC 1.2 or 0 FC 0.83), and a ‘stricter criterion’ of $q < .01$, $|\log_2 \text{FC}| .5$ (approx. FC 1.41, or 0 FC 0.71).

Due to the small number of microarrays, we used a second method of analysis to validate our results based on the Median Absolute Deviation (MAD) of the control genes. In this analysis, each probeset was summarized directly into a single log₂ intensity score for each treatment. We then used the list of 100 probeset id’s designated by Affymetrix as a “normalization control set” (probe numbers 1367452_at-1367551_at on the microarray) to estimate the standard error (SE) in gene expression for each treatment comparison based on the MAD of these control genes. From the SE estimate, paired t-tests were used to calculate P-values of treatment effects for each probeset.

We compared the primary results based on SAM to the results based on MAD using four sets of selection criteria (1, $|\log_2 \text{FC}| \log_2 1.2$ & $P < .05$; 2, $|\log_2 \text{FC}| \log_2 1.2$ & $P < .01$; 3, $|\log_2 \text{FC}| 0.5$ & $P < .005$; 4, $|\log_2 \text{FC}| 0.5$ & $P < .001$). P-values were used rather than q-values because the q-value estimates of MAD results were unreliable (the default method of calculation using the R function “qvalue” gave a nonpositive estimate of 0; see Storey, 2002). By these stated selection criteria, >90% of probes found significant by SAM were also found significant by MAD. However MAD identified between 20% and 65% more probes as significant than did SAM, and is thus less stringent.

The more stringent SAM method was used in a functional analysis based on the Gene Ontologies (GO). The total number of genes meeting the criteria was counted for each GO category, giving sets of genes that were subsets of GO categories that were either up- or down-regulated as a group. For each of these gene subsets, we determined a P-value representing the significance of differential expression of the subset as a whole, as follows: the rank of the log FC of each probeset was determined, and then the rank of the given subset was compared to the rank of all other probesets possessing a GO identifier using the Wilcoxon Rank-Sum test via the R function Wilcox test. The same analysis was done using the Kyoto Encyclopedia of Genes and Genomes (KEGG), however no KEGG category showed significance. The subsets of KEGG pathways that we tested for significance all had a small number of genes. Relaxing the inclusion criteria did yield some significant KEGG gene subsets (not shown). Hence, we attribute the lack of significant KEGG pathways to a combination of the relatively few genes represented in KEGG pathways, and our strict inclusion criteria for individual genes.

Data from two in vivo microarray studies of aging rat hippocampus (Rowe et al., 2007; Kadish et al., 2009) were compared with RNAs differing by age in glial cultures. Gene

symbols were used for comparison rather than probeset id numbers because of the use of different Affymetrix microarrays in these reports.

3. Results

3.1. Age-increase of astrocytic GFAP expression is modified by co-culture with microglia and neurons

Unexpectedly, we found that the age-related increase of astrocytic GFAP expression, as documented in vivo and in vitro (Introduction) was suppressed in mixed glial cultures containing 3:1 astrocytes:microglia. GFAP expression was measured by in situ hybridization in primary glial cultures derived from male rat cerebral cortex of young adult (Y, 3 mo) and old (O, 24 mo). Confirming prior findings (Rozovsky et al., 1998, 2005; Morgan et al., 1999), enriched astrocyte cultures showed the expected age-related increase of GFAP mRNA per cell (Fig. 1a). However, in mixed glia cultures, GFAP mRNA did not show an age-related increase, but was decreased (Fig. 1b). This reversal of direction in GFAP expression is preceded in the switch of GFAP transcriptional responses to steroids from induction to repression, depending on the presence of neurons in co-culture with enriched astrocytes ('transcriptional inversion', Stone et al., 1997) (see Discussion).

The dependence of the direction of GFAP aging changes on the glial cell-type composition was further explored in a separate experiment by Affymetrix microarray profiling (Table 1). In mixed glia without neurons present, GFAP showed a trend towards an age-decrease (−11%, not significant), whereas in mixed glia co-cultured with neurons, GFAP showed a significant age increase (+20%). Apo J (clusterin) mRNA did not show an age change in mixed glia, confirming our prior study (Patel et al., 2004). However, apoJ mRNA showed an age-increase (+8%) in mixed glia co-cultured with neurons. IL-6 showed an age-increase in both culture conditions of over 2-fold, consistent with reports on mixed glia alone (Xie et al., 2003). These findings also extend prior observations on the modulation of GFAP transcription by interactions of astrocytes with neurons and other cell types (Discussion).

3.2. Affymetrix profiles show many neuron-dependent age changes in mixed glia

The Affymetrix profile detected additional neuron-dependent age changes in gene expression (Table 2). Data are presented as Venn diagrams defining subsets of age changes restricted to one set of comparisons (OM vs. YM only and OMN vs. YMN only) and shared changes (Both Age-up or Age-down) observed in glia from different ages grown ± neurons). We did not consider effects of neurons within each age group; thus, the present data cannot resolve whether neuron-suppressed and -enhanced genes were altered within neurons, within glia, or both.

3.2.1. More glial genes show increased expression with aging than decreased

—Two inclusion criteria were evaluated by varying both the q-value and fold-change (FC) (Table 2A). A less strict criterion of $q < 0.05$, $|\log_2 \text{FC}| \geq 1.2$ (approx. FC 1.2 or 0 FC .83) detected several-fold more age changes than a stricter criterion of $q < 0.01$, $|\log \text{FC}| \geq 0.5$ (approx. FC 1.41 or 0 FC 0.71). Across both criteria, there was a consistent excess of Age-up > Age-down (Table 2B). The largest category of age changes was shared (Both Age-up or Both Age-down) across growth conditions. Data for all genes meeting our selection criteria are in Supplemental Tables 1 and 2.

By the stricter criterion, 268 genes scored as Age-up, while 138 were Age-down. Thus, of the 13,746 distinct gene symbols represented on this microarray, 3.0% showed significant age changes at the stricter criterion. Genes corresponding to the Venn diagrams of Table 2 are listed by gene symbols in Supplemental Table 3.

3.2.2. Analysis by functional category (Gene Ontologies, GO)—To examine functional categories, we chose the stricter criterion, which excluded GFAP in the mixed glia-only comparison, but not IL-6. The age changes included 20 GO categories with at least 10 Age-up or 10 Age-down probesets (Table 3). The majority of GO sets showed more Age-up than Age-down, consistent with the overall trend (Table 2B). GO categories with the most age changes (Age-up or Age-down) were ‘protein binding’, ‘membrane’, ‘extracellular region’, ‘integral to membrane’, and ‘extracellular space’, mostly Age-up in co-cultures with neurons. Intermediate numbers of Age-up changes were ‘immune response’, ‘inflammatory response’, and ‘nucleus’. Although ‘Mitochondrion’ was not represented because of too few qualifying probesets, there were statistically significant Age-up and Age-down subsets of ‘mitochondrion’ when analyzed without first applying the exclusion criteria of individual genes (not shown). Overall there were markedly fewer genes in upregulated GO categories in the OM vs. YM comparison than for OMN vs. YMN. These conclusions are tentative because of the small sample sets.

3.3. In vitro and in vivo comparisons

Age changes of gene expression in glial cultures from cerebral cortex were compared with in vivo age changes in the hippocampus reported by Landfield and colleagues (see Introduction). These studies (Rowe et al., 2007; Kadish et al., 2009) differed from ours in their use of the different Affymetrix rat GeneChip, RAE230A, and by a broader criterion for age changes based on statistical significance, but not FC.

Using more stringent criteria, of the 406 genes with age change in vitro (Table 2), 29 genes (about 7%) were shared with in vivo age changes in the same direction in at least one of two studies from the Landfield group (see Table 4 for a list of select genes; information for all genes is in Supplemental Tables 1 and 2). Again, about 2-fold more genes showed age-increases than decreases. Age-up genes relevant to glial inflammatory responses are Cxcl12, Hif1a, Sod3, and Xdh. Age-down genes include Igf2 and Igfbp2 which have notable links to pathways relevant to glial proliferation and longevity (see Discussion). We chose not to model pathways of possible interactions of these shared genes, before verifying specific RNA and protein changes from our glial cultures, as was done by the Landfield group for other genes of interest.

4. Discussion

This study was stimulated by a puzzling observation that appeared to challenge many reports of increased expression of astrocytic GFAP during normal aging, measured as RNA and protein per cell (Introduction). We unexpectedly found age-related decreases of GFAP mRNA per cell by in situ hybridization in mixed glial cultures containing both astrocytes and microglia (3:1). The control culture of enriched astrocytes (<5% microglia or oligodendroglia) showed the expected age increase of GFAP, consistent with prior in vitro studies (Rozovsky et al., 1998, 2005). However, when neurons were added to mixed glia, the age increase of GFAP was restored (this microarray study and Arimoto et al., in prep.).

These phenomena are consistent with the sensitivity of GFAP transcription to interactions with other cell types. For example, GFAP transcription showed opposite responses to estradiol and glucocorticoids in monotypic astrocyte cultures (induction) vs. astrocytes co-cultured with neurons (repression) (Rozovsky et al., 1995; Stone et al., 1998). Moreover, during the estrous cycle, GFAP transcription changed in opposite directions in the hypothalamus and hippocampus (Stone et al., 1998). The present findings add microglia to heterotypic cell interactions that influence GFAP expression. Thus, regional differences in astrocyte GFAP expression during Alzheimer disease could arise from local variations in microglia, as well as neurodegeneration (Coulson et al., 2010; Simpson et al., 2010).

Two further examples from our past studies with neonatal glia show heterotypic cell interactions that modify gene responses. ApoE is induced by estradiol in mixed glia, but did not respond to estradiol in monotypic astrocytes (Stone et al., 1997). Similarly, the cytokine TGF-1 induced apoJ mRNA in pure astrocytes, but decreased apoJ mRNA in mixed glia (Morgan et al., 1995). These findings suggest that heterotypic cell interactions can change the inducibility and the direction of response of diverse genes to physiological modulators, which we have termed 'transcriptional inversion' (Stone et al., 1997). Pilot data indicate the role of AP-1 sites in the GFAP promoter in mediating transcriptional inversion (briefly reported in Stone et al., 1997).

The co-culture of mixed glia with neurons is introduced here as a model in studies of brain aging. Previously we showed that E18 neuron survival and neurite outgrowth was similar with neonatal derived astrocytes or mixed glia (Wong et al., 2009). Mixed glia from young and aged adults (3 and 24 mo) also support E18 neurite outgrowth and show age-impairments of neurotrophic support (Arimoto et al., in prep). The present Affymetrix studies on mixed glial ±neurons are part of the development of this new model to explore the role of neurons in glial gene expression during aging.

Data were analyzed with two levels of stringency according to both statistical significance (q-value) and fold-change (FC). Irrespective of stringency, more RNA sequences showed increased levels with age than were decreased. All culture conditions showed the trend for more Age-up than Age-down changes in glial gene expression. These trends agree with microarray profiles of mouse cerebral cortex, with 3:1 Age-up: Age-down (Loerch et al., 2008), in which astrocytic and oligodendroglial expressed genes are also in excess above chance among Age-up genes.

The co-culture of neurons with mixed glia selectively altered the gene expression profile of many recognized glial genes. Besides the GFAP response to co-culture with neurons, about 25% of age changes (up 28%, 76 genes; down 23%, 32 genes) were only seen in mixed glia-neuron co-cultures. The sensitivity of GFAP to neuronal activity is well known (see Intro). We note the caveat that this experimental design does not resolve the specific contributions of RNA from neurons. Except for GFAP, we also do not know if mRNA changes from age or presence of neurons are translated into protein levels. Further validation is needed of these microarray data for each mRNA and protein.

The glial expressed genes showing neuron-dependent age changes are distributed across the GO categories of Table 3. Future analysis may identify pathways in aging that are shared across inflammatory processes in all tissues (see below), as well as changes in gene expression that are neuron-dependent. The different subset of age changes that was detected in mixed glia alone may define additional pathways of glial response. Thus, we suggest that some glial age changes may arise independently of interactions with healthy or degenerating neurons, e.g. iron deposits which increase with aging in association with myelin degeneration (Bartzokis et al., 2011) could cause local oxidative stress.

The largest GO gene categories of age change were membrane associated. Many of these genes are also closely linked to 'immune', 'inflammatory response', and 'oxidoreductase activity'. This GO cluster is also represented in vivo, where inflammatory processes associated with oxidative stress are increasingly recognized as core processes in aging (Finch, 2010; Morgan and Finch, 2007; Franceschi et al., 2009).

To identify glial age changes relevant to cognitive aging, we compared our findings on rat glial cultures with in vivo age changes in the rat hippocampus reported by Landfield and colleagues (Introduction, Blalock et al., 2003; Rowe et al. 2007; Kadish et al. 2009). These landmark studies identified changes in hippocampal mRNA during middle-age (14 mo) and

old age (24 mo) that correlated with subtle changes in memory performance. In the subsets of genes shared in vitro and in vivo, there were about 2-fold more age-up than age-down. Age-up genes include several with direct links to inflammation. Several Age-down genes are also linked to insulin-like signaling which is implicated in glial proliferation (Chesik et al. 2007, 2010;) and neuronal growth cones (Laurino et al. 2007), as well as to longevity in mouse and invertebrate models (Parrella and Longo, 2010; Panowski and Dillon, 2009) . Future studies will examine the role of glial genes in the pathways proposed by the Landfield group.

We conclude with a historical perspective on the selectivity of changes in gene expression during aging. Until about 1980, prior to the era of recombinant technology, some had concluded that transcription in brain and other tissues becomes progressively impaired during aging (Cutler, 1975; Medvedev et al., 1978; Strehler et al., 1979). These early data did not match a survey of enzyme activities (Finch, 1972) that showed a minority of enzymes (10–20%, depending on tissue) had altered specific activity as measured in whole cell homogenates: in liver and kidney, about 2-fold more enzymes showed increased activity than decreased, consistent with present findings. To further define gene activity changes during aging, we used RNA-driven hybridization kinetics to estimate the transcribed fraction of single-copy DNA (Colman et al., 1980). In two rat strains, the total number of transcripts (sequence complexity) did not differ by age in poly(A) RNA from 3 vs 24 mo old male cerebral cortex, within limits of + 5 %. Provisionally, it may be concluded that adult age changes in genomic expression are highly selective, implying a regulated process. The present model of mixed glial cultures further documents the selectivity of changes in gene expression. Analysis of glialneural interactions during later stages of brain maturation may reveal the basis for the onset of cognitive declines and synaptic attrition, which soon follows sexual maturation (Blalock et al., 2003; Finch, 2009; McArdle, 2002).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

Aging increases gene expression in mixed glia

Fig. 1.

In situ hybridization assay of GFAP mRNA in cultured glia from rat cerebral cortex of two ages: Young (3 mo) and Old (24 mo) male rats. Grains per cell, from 250 individual cells per slide, 3 slides per culture type and age, was expressed as % young, Mean $\pm$ SEM. (A) In enriched astrocyte cultures (>95%), GFAP mRNA was 3-fold higher in Old than Young (** $p < 0.03$). (B) In mixed glia (3:1, astrocytes: microglia), GFAP mRNA levels were 0.40-fold lower in Old than Young (* $p < 0.05$).

Table 1

Affymetrix gene expression levels (average normalized intensity) for Apo J (clusterin), GFAP and IL-6. The percentages given represent percentages increased/decreased (+/-) with age: (Y-O)/Y; NS, not significant.

	ApoJ	GFAP	IL-6
YM OM	54.98 56.06 +2%, $q = 0.42$ (NS)	55.06 49.44 -11%, $q = 0.10$ (NS)	76.81 223.09 +190%, $q \ll 0.01$
YMN OMN	52.50 56.50 +8%, $q = 0.03$	96.98 116.79 +20%, $q \ll 0.01$	55.86 160.56 +187%, $q \ll 0.01$

Table 2

Numbers of age changes (O/Y) in mixed glia (M) grown with or without neurons (N) by two inclusion criteria: relaxed, $q < 0.05$, $|\log FC| \geq 1.2$ (approx. FC 1.2 or 0 FC .83); stricter, $q < .01$, $|\log FC| \geq .5$ (approx. FC 1.41 or 0 FC .71).

A. Venn diagrams for the numbers age changes (O, Y) in gene expression in mixed glia (M) grown $\pm$ neurons (N)			
Age-up,			
	<i>OM>YM only</i>	<i>Both Age-up</i>	<i>OMN>YMN only</i>
Relaxed criterion	214	456	376
Stricter criterion	50	142	76
Age-down			
	<i>OM<YM only</i>	<i>Both Age-down</i>	<i>OMN<YMN only</i>
Relaxed criterion	118	184	137
Stricter criterion	38	68	32

B Summary of proportions of categories		
<i>Age-up:Age-down</i>	Relaxed criterion	Stricter criterion
<i>[OM>YM only]:[OM<YM only]</i>	1.81	1.32
<i>Both Age-up :Both Age-down</i>	2.48	2.09
<i>[OMN>YMN only]:[OMN<YMN only]</i>	2.74	2.38

Table 3

GO gene sets that contained 10 age-increased genes OR 10 age decreased genes ($q < 0.01$, $\log FC > 0.5$) in 1 of the comparisons. No KEGG pathways met the criteria. p values were calculated for the gene sets by Wilcoxon scoring (Methods). $p < 0.01$ for all indicated gene sets except those with either 0 or 1 gene, for which a gene-set p value is not applicable.

Pathway	OM>YM	OM<YM	OMN>YMN	OMN<YMN
calcium ion binding	7	6	14	5
chemokine activity	6	0	14	0
chemotaxis	5	0	11	0
cytoplasm	8	4	17	5
endoplasmic reticulum	8	2	12	2
extracellular region	14	5	34	8
extracellular space	12	2	24	3
hydrolase activity	5	2	15	2
immune response	7	0	22	0
inflammatory response	6	2	16	4
integral to membrane	12	17	33	18
membrane	22	22	44	23
metal ion binding	7	1	18	4
multicellular organismal development	5	4	10	4
nucleus	4	5	20	13
oxidoreductase activity	9	0	14	1
protein binding	27	11	48	21
proteolysis	7	1	10	2
receptor activity	7	4	10	4
zinc ion binding	7	2	13	5

Table 4

Select gene expression age changes from Table 2 (stricter criteria) that changed in the same directions in the aging rat hippocampus in studies from the Landfield group, as denoted by: R, Rowe et al. (2007) RS, Supplemental tables of Rowe; K, Kadish et al. (2009), KS, Supplemental tables of Kadish. All entries below showed significant age changes under both glial culture conditions (with- or without neurons) except for GFAP. Complete information is in Supplemental Tables 1 and 2.

<i>Age-up</i>
Cxcl12 (R,KS): chemokine (C-X-C motif) ligand 12; S100B/RAGE induction
Enpp2 (RS): ectonucleotide pyrophosphatase/phosphodiesterase 2; extracellular lysophospholipase D.
Gfap (R): glial fibrillary acidic protein
Hif1a (RS): hypoxia inducible factor 1a; transcription factor, regulates inflammatory cytokines in astrocytes
Magi3 (KS,RS): membrane associated guanylate kinase associated with E-cadherin base cellular contacts of astrocytes
Pla2g4a (K,RS): phospholipase A2, group IVA (cytosolic, Ca-dependent); HNE upregulates and phosphorylates cPLA(2) in microglia via ERK and p38 MAPK.
Pld1 (K,RS): phospholipase D1, phosphatidylcholine-specific, with phosphatidylinositol-binding PX and PH domains; activates PLD downstream of protein kinase C; may be required for astroglial proliferation.
Prss23 (RS) serine protease 23
Rnase4 (KS)
Sod3 (KS): superoxide dismutase 3, extracellular
Xdh (KS) xanthine dehydrogenase; generates superoxide
<i>Age-down</i>
Crym (RS): mu-crystallin, identical to cytosolic-T3-binding protein;
Igf2 (KS): insulin-like growth factor 2 (somatomedin A)
Igf2bp2 (K, RS): insulin-like growth factor binding protein 2,
Nnat (RS): neuronatin; CA1 dendritic protein; regulates dendritic Ca ⁺⁺
Ptprd (K, RS): protein tyrosine phosphatase, receptor type, D
Serpini1 (K): serpin peptidase inhibitor, clade I (neuroserpin),