

## Genomic transcriptional profiling in LOU/C/Jall rats identifies genes for successful aging

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**Abstract** Inbred LOU/C/Jall rats are currently described as a model of successful aging. These rats have a longer healthy median lifespan than many other strains, do not develop obesity, diabetes, or tumor and more importantly they do not show cognitive decline with aging. This is the first study to examine gene expression changes in the inbred LOU/C/Jall rat hippocampus and frontal cortex. Microarray data from animals aged 5 and 26 months were compared to that obtained from the classical Wistar rat strain to potentially identify only the genes associated with successful aging. We have thus identified a set of 15 genes in the hippocampus and 70 genes in the frontal cortex that could be grouped into several clearly delineated clusters of highly correlated genes associated with a diversity of biological processes, including regulation of plasticity, inflammatory response, metabolic, catabolic and homeostatic processes,

and transcription. Such a multiplicity of gene networks and diversity of biological functions were not observed in the Wistar rat strain. The gene expression profiles identified in aged the LOU/C/Jall rats' hippocampus and frontal cortex should be related to their intact cognitive abilities, such as those assessed through spontaneous alternation.

**Keywords** Microarray · Neuronal network · Hippocampus · Frontal cortex · LOU/C/Jall rats

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### Introduction

Aging is associated with numerous tissue alterations such as a decrease in DNA repair mechanisms' efficiency (Lombard et al. 2005), mitochondrial oxidative stress production (Cortopassi and Arnheim 1990), the downregulation of synaptic proteins synthesis involved in the structural plasticity of axons and dendrites (Blalock et al. 2003), and an increase in neuroinflammatory responses (Godbout and Johnson 2006). Aging affects the central nervous system as a whole, but age-related differences are most often highly circumscribed and confined to specific regions, in particular to the hippocampus and the frontal cortex (Rapp and Heindel 1994; Raz 2004; Small 2001). Interestingly, these regions that are highly involved in high cognitive functions such as learning and memory are affected in neurodegenerative disorders. In recent years, numerous microarray studies have been conducted on the aging brain to identify genes and biological pathways related to the aging process. Most of them focused on transcriptional changes occurring in the aging hippocampus. Changes in genes expression associated with lipid catabolism, proteolysis, cholesterol transport, ion conductivity, inflammation, and myelinogenesis have thus been

observed (Blalock et al. 2003, 2010; Burger et al. 2007, 2008; De Magalhães et al. 2009; Haberman et al. 2011; Kadish et al. 2009; Rowe et al. 2007).

Rodents have been extensively used in studies on aging, including standard rat strains such as Fischer 344, Wistar, Sprague-Dawley and Long-Evans. LOU/C/Jall rats, an inbred strain of Wistar origin, have recently been described as a model of successful aging (Alliot et al. 2002; Boghossian et al. 2002; Dubeau et al. 2011; Garait et al. 2005). Rats from this strain have a longer healthy median lifespan than many other strains, i.e. 29 months in males and 33–34 months in females (Alliot et al. 2002). They do not develop severe pathologies with age, such as obesity, diabetes, or tumors. The most interesting characteristic of the LOU/C/Jall strain is the absence of cognitive decline with advanced age. In an object recognition task, Kollen et al. (2010) demonstrated that 24-month-old LOU/C/Jall rats displayed significantly higher retention performances compared to other strains of rats. Interestingly, these preserved behavioral abilities in aged LOU/C/Jall rats are correlated with intact hippocampal functioning, which is involved in memory formation including the expression of *N*-methyl-D-aspartate receptor-mediated long-term potentiation (Kollen et al. 2010; Turpin et al. 2011).

The aim of the present study is to gain new insights into the aging process and more specifically to potentially identify a set of genes that are involved in successful aging. To this end, we have undertaken a genomic wide expression analysis in young and aged LOU/C/Jall rats. The resulting genes list was compared to the one obtained from young and aged Wistar rats. Studies focused on the hippocampal formation and the frontal cortex, two structures known as being particularly vulnerable to the effects of aging (DeVito and Eichenbaum 2010). They are involved in high cognitive function, such as learning and memory that can be assessed through spontaneous alternation in Y-maze. Both young and aged LOU/C/Jall and Wistar rats were submitted to spontaneous alternation in order to evaluate their performances in a behavioral task involving the cognitive abilities related to spatial working memory (Lalonde 2002).

## Materials and methods

### Animals

Male Wistar (Janvier, France) and LOU/C/Jall rats (derived from the inbred Lou/C strain, originally imported to Louvain and bred at the Complexe scientifique des Cégeaux, Université Blaise-Pascal, Aubière, France) were used in the experiments. Rats were housed in standard conditions, i.e., in groups of 2–3 rats per cage under 12 h light/12 h dark conditions with ad libitum access to food and water. Every

effort was made to minimize animal suffering and to reduce the number of rats used.

Four groups of rats were constituted according to their age: young adults (5 months old;  $N = 13$  Wistar and  $N = 14$  LOU/C/Jall) and aged rats (26 months old;  $N = 11$  Wistar and  $N = 14$  LOU/C/Jall). Four rats per group were used for the microarray study and 7–10 rats per group for behavioral design. Experiments were conducted in compliance with the international guidelines on the ethical use of animals (personal authorization number: 14-101).

### Tissue collection and RNAs extraction

Rats were anesthetized with isoflurane [5 % in  $N_2O/O_2$  (2/1)], decapitated, and the brain was removed. Their hippocampus and frontal cortex were dissected out in two hemi-structures and immediately frozen in liquid nitrogen. The dissected tissues were immediately homogenized and purified with a mirVana miRNA Kit (AB Applied-Ambion, France) according to the manufacturer's protocol. RNA concentration was determined with UV spectrophotometry. RNA integrity was assessed with a Bioanalyzer 2100 (Agilent Technologies, France) and only high quality RNA was used for further analysis (average RNA integrity number was  $9.24 \pm 0.07$ ).

### Preparation of samples and microarray assay

Sample amplification, labeling, and hybridization essentially followed the one-color microarray-based gene expression analysis (low input quick amp labeling) protocol (version 6.5, May 2010) recommended by Agilent Technologies. Thereby, 100 ng of each total RNA sample was reverse transcribed into cDNA using oligo dT-T7 promoter primer. Labeled cRNA was synthesized from the cDNA. The reaction was performed in a solution containing a dNTP mix, cyanine 3-dCTP, and T7 RNA Polymerase, and incubated at 40 °C for 2 h. Qiagen's RNeasy mini spin columns were used for purification of the amplified cRNA samples. cRNA was quantified using NanoDrop Spectrophotometer version 3.2.1. 600 ng of cyanine 3-labeled, linearly amplified cRNA was used for hybridization. Hybridization was performed in a hybridization oven at 65 °C for 17 h at 10 rpm in SurePrint G3 Rat GE microarray slides ( $8 \times 60$  K G4853A, Agilent Technologies, Santa Clara, CA) containing 60,000 oligonucleotide probes. Hybridized microarray slides were then washed according to manufacturer's instructions and scanned using an AgilentMicroarray Scanner, using the Agilent Feature Extraction Software (Agilent Technologies). The microarray data are available from the Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) under the series accession number GSE36722.

## Microarray data analysis

Quantification files derived from the Agilent Feature Extraction Software were analyzed using the AgiND package (<http://www.tagc.univmrs.fr/AgiND>). We also used the AgiND R package for quality control and normalization. Quantile methods and a background correction were used for data normalization. Gene expression data were then imported into Excel (Microsoft®) for filtering and statistical analyses. A Welch *t* test was used independently for each gene. Probe sets were considered to be statistically significant at  $P < 0.005$ , with a false discovery rate (FDR) less than 1 %. The cut-off value for gene up- or down-regulation was set at 0.5 in the log<sub>2</sub> scale (corresponding to a 1.4-fold change). The genes of interest were those satisfying the following three criteria: (1)  $P$  value  $< 0.005$ , (2)  $FDR < 0.01$  and (3) absolute LogR  $\geq 0.5$ , so that the genes included may be more likely to result in significant physiological effects. The expression data of the genes of interest were used to build networks of genes using the Trixy software (<http://www.tagc.univ-mrs.fr/bioinformatics/trixy.html>) as described previously (Paban et al. 2010, 2011). The algorithm basically relies on two user-controlled parameters: the correlation coefficient (Pearson) and the clustering coefficient, which can be set between 0 and 1. A graph is built with one node for each gene and a link between genes with absolute correlation equal to the correlation threshold. A subgraph is then displayed by showing only those nodes with a clustering coefficient or curvature equal to the clustering threshold (Eckmann and Moses 2002; Watts and Strogatz 1998). The thresholds for these two coefficients were set at their highest value, 1. Note that clusters of high clustering coefficient are highly non-random structures (Collet and Eckmann 2002). This makes it possible to identify the genes that co-vary perfectly, or nearly perfectly, together or oppositely and the genes with maximum links, i.e., the genes with a high density of ties (for details on the algorithm, see Rougemont and Hingamp 2003). Functional profiling of these gene networks was performed on the Onto-Express website (<http://www.vortex.cs.wayne.edu/projects.htm>) (Draghici et al. 2003; Khatri et al. 2002), which allowed us to thoroughly characterize sets of functionally related genes based on GO categories. Over-represented GO categories were identified using the hypergeometric distribution. Only those GO categories with a corrected  $p$  value  $< 0.05$  were considered.

## Real-time quantitative RT-PCR

In order to further confirm the reliability of the array data, the mRNA levels of a subset of genes were quantified with qRT PCR. These genes were selected on the basis of different expression characteristics, including up- and

down-regulated expression and possessed different biological functions. qRT PCR assays were conducted according to the manufacturer's instructions (Ab Applied, France) and as previously described (Paban et al. 2010, 2011). The expression analyses of A2m (NM\_012488), Aadat (NM\_017193), Acn9 (NM\_001047914), Actg2 (NM\_012893), Arc (NM\_019361), C3 (NM\_016994), Ccnd1 (NM\_171992), Cox6a2 (NM\_012812), Dusp1 (NM\_053769), Egr2 (NM\_053633), Fcgr2b (NM\_175756), Fos (NM\_022197), Frzb (NM\_001100527), Gadd45g (NM\_001077640), Gfap (NM\_017009), Gria2 (NM\_017261), Grip2 (NM\_138535), Grp (NM\_133570), Hcn1 (NM\_053375.1), Junb (NM\_021836), Nef3 (NM\_017029), Nmbr (NM\_012799), Nr4a1 (NM\_024388), Pcsk1 (NM\_017091), S100a4 (NM\_012618), Scn7a (NM\_031686), St8sia5 (NM\_213628) genes were performed using a LightCycler 480 Instrument with software version 1.5.0 (Roche, Meylan, France). Each reaction was run in duplicate in a final volume of 25  $\mu$ l containing 12.5  $\mu$ l of SYBR Green 2 X Master Mix (Roche Diagnostics, Mannheim, Germany), 300 nmol/l of each primer, and 5  $\mu$ l of cDNA. PCR programs were carried out as follows: 1 cycle at 95 °C for 5 min and 45 cycles of amplification (95 °C for 10 s, 60 °C for 30 s, and 72 °C for 15 s). The crossing point (Ct) of each set of primers was determined and compared to the Ct of the peptidylprolyl isomerase A (Ppia, NM\_017101.1) mRNA used as endogenous controls. Data were then imported into Excel (Microsoft) for log<sub>2</sub> transformation and statistical analyses. The effect of age on relative expression was assessed with a Welch *t* test for each rat strain and cerebral structure. Linear regression analysis was then performed between qRT PCR and microarray data.

## Spontaneous alternation

Immediate spatial working memory performances were assessed by recording spontaneous alternation behavior in a single-session Y-maze test (as performed in Bouet et al. 2010, 2012). The maze consisted of three equally spaced arms (50 cm long, 15 cm wide, walls 32 cm high) made of gray-painted wood. Each rat was placed at the end of one arm and allowed to explore the maze freely during a 5-min session. The number and the sequence of arm entries were recorded. An arm entry was scored when all four feet crossed into the arm. Alternation behavior is defined as consecutive entries into all three arms. The percentage of alternation was calculated as a memory index by the (number of alternation/maximal theoretical number of alternation)  $\times 100$ . The number of visited arms was used as an index of global activity.

Data were analyzed with a two-way analysis of variance (ANOVA) with age and strain as independent factors, followed by post hoc multiple comparisons tests (Fischer's

PLSD) (Statview®). Univariate *t* test was used to compare the working memory performances to a reference value (50 % alternation corresponding to a random exploration of the Y-maze).

## Results

Changes in gene expression during aging occurred in the two rat strains no matter what the brain region was. On the whole, 75 genes were significantly changed in Wistar rats and 90 genes in LOU/C/Jall rats. Interestingly, the selected genes were mostly up-regulated with aging in LOU/C/Jall (80 % of genes) compared to Wistar rat brains, in which the selected genes were equally up- and down-regulated (53 and 47 % of genes, respectively). The full set of 75 and 90 genes is shown in Table S1 (supplementary material).

### Age-related gene profiling in the hippocampal and cortex frontal regions of Wistar rats

In this classical strain of rats, aging in the hippocampus induced an expression modification of 16 genes identified in knowledge banks (including PubMed, EASE, NetAffx). Among them, ten were up-regulated and six down-regulated (Table 1). Networks of co-expressed genes can be seen in Fig. 1a. When the correlation and clustering coefficients were set at 1, only one cluster of seven genes could be identified. Functional profiles are reported in Fig. 1a.

In the frontal cortex, 59 genes were identified in knowledge banks, of which 29 were up-regulated and 30 down-regulated (Table 1). Networks of co-expressed genes can be seen in Fig. 1b. Two clusters of highly correlated genes were clearly delineated. Functional profiles are reported in Fig. 1b.

### Age-related gene profiling in the hippocampal and cortex frontal regions of LOU/C/Jall rats

In the hippocampus, 17 genes were differentially expressed ( $P < 0.005$ ,  $FDR < 0.01$  and  $\text{LogR} \geq 0.5$ ) and identified in knowledge banks. Among them, 16 were up-regulated and only 1 was down-regulated (Table 1). The genes associated with successful aging in the hippocampus are those potentially uniquely expressed in the LOU/C/Jall rats. To identify these genes, a Venn diagram was built from the selected genes in Wistar and LOU/C/Jall rats. It then allowed us to highlight the presence of 15 genes only expressed in LOU/C/Jall rats (Table 1), only 2 genes being common to both strains, C3 and Fcgr2b. Within the top 10 of genes up-regulated by LOU/C/Jall hippocampus, the highest ranked annotated were Lgals3 (Galectin-3; fold change: 1), and S100a4 (Protein S100-A4; fold change: 1), and Bcl3 (B cell CLL/lymphoma 3; fold change 1), seen from Table 2. Only

**Table 1** Number of genes significantly expressed in the hippocampus and frontal cortex of Wistar and LOU/C/Jall rats

Wistar rat	LOU/C/Jall	Venn diagram
<b>Hippocampus</b>		
Total genes = 16	Total genes = 17	
Up-regulated genes = 10	Up-regulated genes = 16	
Down-regulated genes = 6	Down-regulated genes = 1	
<b>Frontal cortex</b>		
Total genes = 59	Total genes = 73	
Up-regulated genes = 29	Up-regulated genes = 56	
Down-regulated genes = 30	Down-regulated genes = 17	

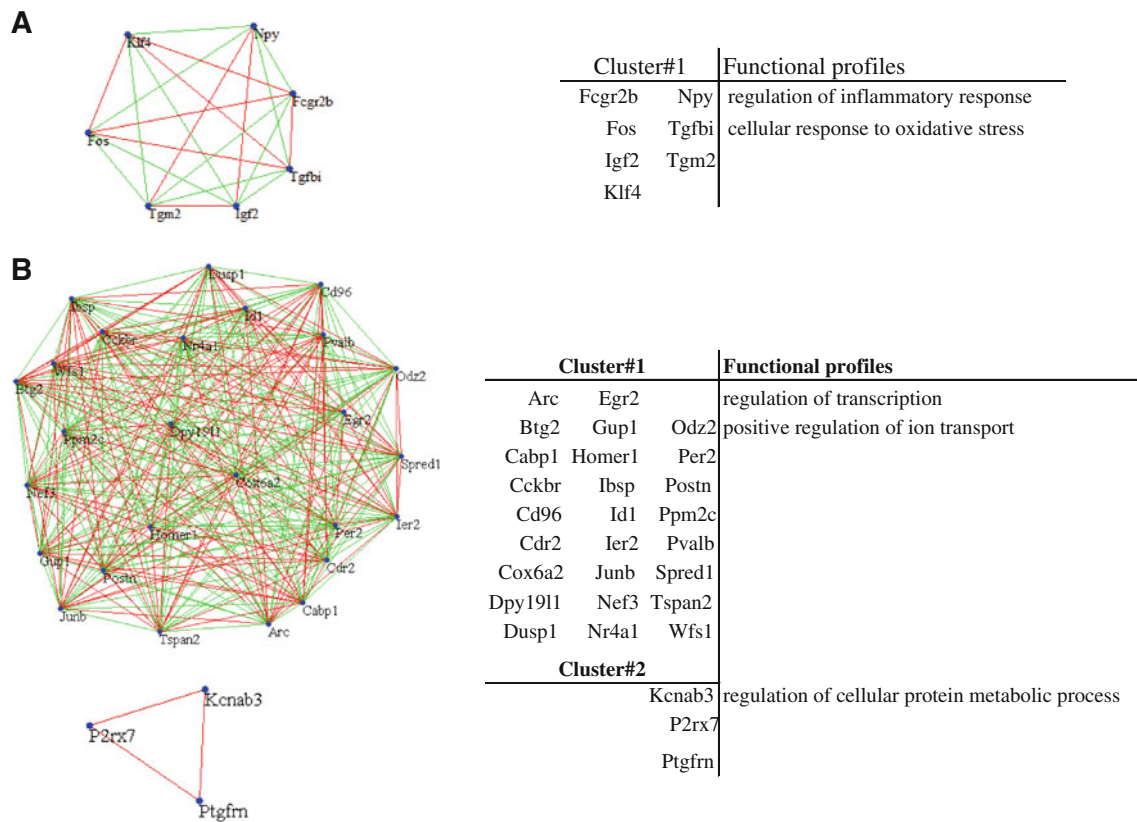
The total number and the number of up- and down-regulated genes in each rat strain and brain region are indicated. A Venn diagram was built from these selected genes in Wistar and LOU/C/Jall rats' hippocampus and frontal cortex in order to potentially identify genes uniquely expressed in the LOU/C/Jall rats. The common genes, i.e., the number of genes affected by aging no matter what the rat strain was, are indicated in the center

one gene was down-regulated, *St8sia5* (fold change:  $-1.19$ ). Networks of co-expressed genes are displayed in Fig. 2a. When the correlation and clustering coefficients were set at 1, three clusters could be identified. Functional profiles are reported in Fig. 2a.

In the frontal cortex, aging induced an expression modification of 73 genes ( $P < 0.005$ ,  $FDR < 0.01$  and  $\text{LogR} \geq 0.5$ ). Most of them were up-regulated ( $N = 56$  genes). Only 17 genes were down-regulated (Table 1). The Venn diagram identified 70 genes potentially uniquely expressed in the LOU/C/Jall rat's cortex. Note that only three genes differentially expressed in the frontal cortex were common to both rat strains, *C3*, *Dusp*, and *Frzb*. Within the top ten of genes up-regulated by healthy aging, the highest ranked annotated were *A2m* (Alpha-2-macroglobulin; fold change: 1,2) and *S100a4* (Protein S100-A4; fold change: 1,1) (Table 3). Within the top ten down-regulated genes, the two genes with the highest fold changes were *Nmbr* (Neuromedin-B receptor; fold change:  $-1.19$ ) and *Sla* (SRC-like-adaptor; fold change:  $-0.97$ ). Networks of co-expressed genes are shown in Fig. 2b. Height clusters were identified. Functional profiles are reported in Fig. 2b.

### qRT PCR analysis

We then validated our microarray results with qRT PCR using primers and probes for a subset of genes. These genes



**Fig. 1** Networks of selected genes in Wistar rats' hippocampus (a) and frontal cortex (b) using Trixy software (<http://www.tagc.univ-mrs.fr/bioinformatics/trixy.html>). This provides a graphical representation of gene clusters. Nodes are genes, and links symbolize co-expression. The red line indicates positive correlations and the

green line negative correlations. For more clarity, gene symbols are provided in tables. The functional categories identified by Onto-Express (<http://www.vortex.cs.wayne.edu/projects.htm>) were noted for each cluster

were chosen based on their biological relevance and for their wide range of positive and negative Log-ratio (ranging from  $-1.49$  to  $+2.04$ ). The mRNA levels of 17 genes from old Wistar rats were compared to the ones measured in young rats. In the hippocampus, *Act2*, *Arc*, *Ccnd1*, *Fos*, *Gadd45g*, *Gfap*, and *Grip2* genes showed up-regulation in aged rats (Fig. 3a) whereas *Gria2*, *Hcn1*, and *Pcsk1* genes were down-regulated. In the frontal cortex, *Arc*, *C3*, *Egr2*, *Fos*, *Gfap*, *Grp*, *Junb*, and *Nr4a1* genes were up-regulated and *Cox6a2*, *Frzb*, and *Nef3* were down-regulated in aged rats (Fig. 3c). The mRNA levels of 12 genes from old LOU/C/Jall rats were compared to the ones measured in young ones (Fig. 4). In the hippocampus, *Aadat*, *C3*, *Fcgr2b*, *Gfap*, *S100a4*, and *Scn7a* genes were up-regulated whereas the *St8sia5* gene was down-regulated in aged rats (Fig. 4a). In the frontal cortex, *A2m*, *Acn9*, *C3*, *Fcgr2b*, *Gfap*, *Grip2*, and *S100a4* genes were up-regulated and *Dusp1*, *Nmbr* genes were down-regulated in old rats (Fig. 4c).

Therefore, gene expression changes measured with qRT PCR correlate strongly with changes found through microarray analysis in rats' hippocampus (Fig. 3b, d) and frontal cortex (Fig. 4b, d) in both strains.

## Spontaneous alternation

The results are shown in Fig. 5. ANOVA revealed a significant age and strain effect on alternation percentages (respectively  $P = 0.01$  and  $P = 0.05$ ), but no interaction. Interestingly, univariate *t* test revealed that Wistar rats aged 25 months were the only group that could not reach an alternation percentage significantly different from 50 %, indicating deficits in the alternation task. For the 3 other groups, alternation percentages were significantly above 50 % ( $P < 0.0001$ ,  $P = 0.0002$ ,  $P = 0.0004$  for young Wistar, young LOU/C/Jall, and old LOU/C/Jall, respectively), indicating significant performances in immediate spatial working memory.

## Discussion

This is the first study to examine gene expression changes in the inbred LOU/C/Jall rats, which have recently been described as a model of successful aging (Alliot et al. 2002; Boghossian et al. 2002; Dubeau et al. 2011; Garait et al.

**Table 2** Top 11 of most up- and down-regulated genes in the hippocampus of LOU/C/Jall rats

Gene symbol	Gene name	LogR	GO biological process description
<i>Up-regulated gene</i>			
Lgals3	Galectin-3	1.0	Immune system process; induction of apoptosis; cell adhesion
S100a4	Protein S100-A4	1.0	Immune response; macrophage activation; cell cycle; intracellular signaling cascade; cell motion; signal transduction; response to stimulus
Bcl 3	B cell CLL/lymphoma 3	1.0	Cellular component organization
Pbld	Phenazine biosynthesis-like domain-containing	0.8	Metabolic process
Gpnmb	Transmembrane glycoprotein NMB	0.8	Cell adhesion
Scn7a	Scn7a	0.7	Neuronal action potential propagation; cation transport
Birc7	Baculoviral IAP repeat-containing 7	0.7	Metal ion binding
Spp1	Osteopontin	0.8	Immune system process; cell adhesion; cellular component morphogenesis
Lsp1	Lsp1	0.8	Immune system process; signal transduction; cell motion; signal transduction
<i>Down-regulated gene</i>			
St8sia5		-0.5	Carbohydrate metabolic process; lipid metabolic process; protein metabolic process

Gene symbols, names, and LogRatio are provided. A positive LogR indicates an increase and negative score a decrease in gene expression in aged rats (26 months old) compared to young rats (5 months old)

2005; Kollen et al. 2010; Turpin et al. 2011). We identified a set of genes potentially uniquely associated with health during the aging process, among which 15 genes are in the hippocampus and 70 genes in the frontal cortex. It may be argued that these differences only result from the determination at an earlier timepoint in LOU/C/Jall rats since these animals have a longer lifespan than the Wistar ones. However, it was shown that the expression of synaptic plasticity in neuronal networks as well as astrocyte phenotype was preserved in aged LOU/C/Jall rats not only at 24 months old, which corresponds to the median life span

of Wistar rats, but also at 29 months old, i.e. their own median life span, indicating that LOU/C/Jall rats do not display a delayed but rather a successful brain aging (Kollen et al. 2010; Turpin et al. 2011). Interestingly, compared to the meta-analysis of age-related gene expression profiles performed by De Magalhães et al. (2009), among the 85 genes significantly expressed in the hippocampus and frontal cortex of LOU/C/Jall aged rats, only 9 genes overlapped. These included A2m, C3, Chrn1, Fcgr2b, Gfap, Gpnmb, Lgals3, S100a4, and Spp1 genes, suggesting that these expression changes were common with aging in brain tissue. Some specificity should exist since they have not been identified in Wistar rats as shown in the present study and by others such as Stranahan et al. (2010) in Wistar rats, Burger et al. (2008) and Blalock et al. (2003) in Fisher-344 rats.

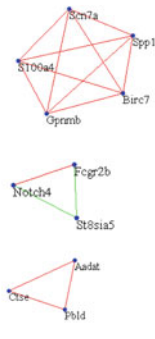
In consistence with the results from Kollen et al. (2010), our behavioral study showed that LOU/C/Jall rats preserved memory capacities in advanced age, as underlined by the preservation of alternation percentages between 5 and 25 months. On the contrary, aged Wistar rats had decreased performances, as demonstrated by the absence of significant differences in 50 % of them. Interestingly, the increased variability in aged Wistar rats indicates that some animals from this group are more affected than others, while in aged LOU/C/Jall rats, the small variability indicates that their performances are preserved and that individual performances are closer to one another. Such a decrease in performances had already been reported in aged as well as middle-aged Wistar rats (Vila-Luna et al. 2012), but never been studied in LOU/C/Jall rats when rats were submitted to spontaneous alternation behavior.

From our microarray study, the most striking result is the organization of genes in several clearly delineated clusters in LOU/C/Jall strain rats. This phenomenon was noted no matter what the brain region was—hippocampus or frontal cortex. Such a multiplicity of gene networks was not observed in the Wistar strain. In addition, in contrast to Wistar rats, most differentially expressed genes were up-regulated in LOU/C/Jall animals.

In the hippocampus, among the 15 genes selected, only one was down-regulated. It belongs to one of the three clusters. St8sia5 associated with Notch4 and Fcgrb could be involved in the regulation of inflammatory responses. Alterations in immune response and in mitochondrial processes have been reported in the whole brain, frontal cortex, and hippocampus of aged rodents (Cocco et al. 2005; Navarro et al. 2008). Note however that the genes involved in these processes were different according to the strains, Wistar versus LOU/C/Jall rats. Altogether, one may suggest that successful aging, such as described in LOU/C/Jall rats, is related to the expression of specific genes, different from those existing in classical strains, which

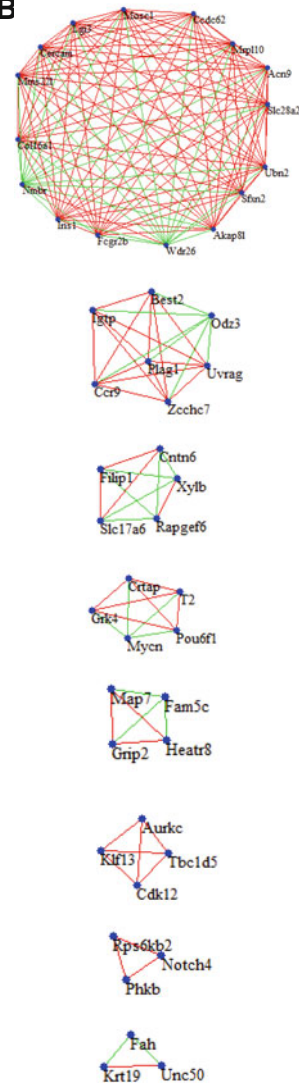
**Fig. 2** Networks of selected genes in LOU/C/Jall rats' hippocampus (a) and frontal cortex (b) using Trixy software. Gene symbols and functional categories via Onto-Express were noted for each cluster. Details are given in Fig. 1

**A**



Cluster#1	Functional profiles
Birc7 Scn7a	regulation of collateral sprouting
Gpnmb Spp1	axon regeneration
S100a4	
Cluster#2	
Fcgr2b	regulation of inflammatory response to antigenic stimulus
Notch4	
St8sia5	
Cluster#3	
Aadat	amino acid catabolic process
Ctse	
Pblid	

**B**



Cluster#1	Functional profiles
Acn9 Mms22l	regulation of phagocytosis
Akap81 Msc1	inflammatory response to antigenic stimulus
Ccdc62 Mrp110	
Cercam Nmb	
Col16a1 Sfxn2	
Fcgr2b Slc28a2	
Ins1 Ubn2	
Lgi3 Wdr26	
Cluster#2	
Best2	homeostatic process
Ccr9 Plag1	
Igt Uvrag	
Odz3 Zcchc7	
Cluster#3	
Cntn6	regulation of neurotransmitter levels
Filip1 Slc17a6	uptake
Rapgef6 Xylb	
Cluster#4	
Crtap	receptor metabolic process
Grk4 T2	
Mycn Pou6f1	
Cluster#5	
Fam5c Heatr8	nucleus organization
Grip2 Map7	
Cluster#6	
Aurkc Klf13	regulation of transcription
Cdk12 Tbc1d5	
Cluster#7	
Notch4	endothelial cell morphogenesis
Phkb	
Rps6kb2	
Cluster#8	
Fah	catabolic process
Krt19	
Unc50	

enables them to associate to others in small well-organized groups. The cluster including up to five genes, notably Birc7 (Yang et al. 2010), Spp1 (Ellison et al. 1999), and S100a4 (Senolt et al. 2006) is of particular interest. Functional profiling indeed indicated that it could be associated

with collateral sprouting and axon regeneration. Interestingly, the genes belonging to this cluster were highly up-regulated in LOU/C/Jall rats, suggesting a stimulation of such processes in the hippocampus of those rats. Similarly, focusing on functional rather structural plasticity, it has

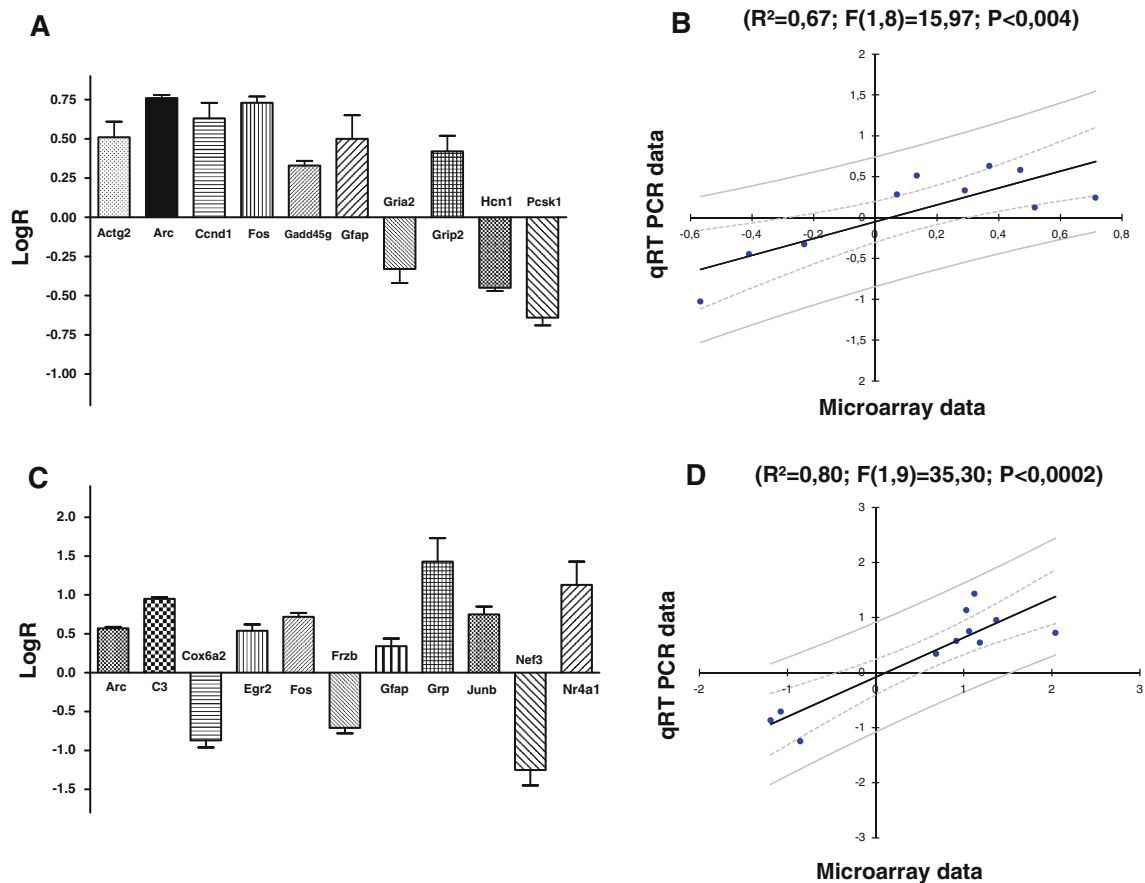
**Table 3** Top 20 of most up- and down-regulated genes in the cortex frontal of LOU/C/Jall rats

Gene symbol	Gene name	LogR	GO biological process description
<i>Top 10 most up-regulated genes</i>			
A2m	Alpha-2-macroglobulin	1.2	Signal transduction; protein metabolic process; signal transduction; cell–cell signaling; response to stimulus
S100a4	Protein S100-A4	1.1	Immune response; macrophage activation; cell cycle; intracellular signaling cascade; cell motion; signal transduction; response to stimulus
C3	Complement C3c alpha' chain fragment 2	1.1	Complement activation; cell–cell signaling; protein metabolic process; signal transduction; response to stimulus
Gimap9	Gimap9	1.0	Immune system process
Heatr8	HEAT repeat-containing 8	1.0	Cellular process
Sdpr	Serum deprivation-response protein	1.0	Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
Igtp	Interferon gamma induced GTPase	0.9	Immune system process
Lipogenin	Lipogenin	0.9	Metabolic process
Acn9	Protein ACN9 homolog, mitochondrial	0.8	Carbohydrate metabolic process
Slc28a2	Sodium/nucleoside cotransporter 2	0.8	Cation transport; nucleobase, nucleoside, nucleotide and nucleic acid transport; nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
<i>Top 10 most down-regulated genes</i>			
Nmbr	Neuromedin-B receptor	−1.19	Immune system process; blood circulation; neurological system process; induction of apoptosis; cell surface receptor linked signal transduction; intracellular signaling cascade; carbohydrate metabolic process; signal transduction; cellular glucose homeostasis; regulation of vasoconstriction
Sla	SRC-like-adapter	−0.97	Immune system process; cell surface receptor linked signal transduction; cell motion; signal transduction; cellular defense response
Mmp14	Matrix metalloproteinase-14	−0.92	Protein metabolic process
Slc17a6	Vesicular glutamate transporter 2	−0.88	Cation transport; phosphate transport; extracellular transport; carbohydrate transport; intracellular protein transport; endocytosis; vitamin transport; phosphate metabolic process; carbohydrate metabolic process
Frzb	Frzb	−0.79	Cell surface receptor linked signal transduction; cell–cell signaling; signal transduction
Dusp1	Dual specificity protein phosphatase 1	−0.75	Immune system process; intracellular signaling cascade; carbohydrate metabolic process; nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; lipid metabolic process; protein metabolic process; cell cycle; signal transduction; response to
Fam5c	Family with sequence similarity 5, member C	−0.75	Cell cycle
Filip1	Filamin A interacting protein 1	−0.68	Cellular component morphogenesis; cellular component organization
Atp2a3	Sarcoplasmic/endoplasmic reticulum calcium A	−0.62	Cation transport; lipid transport; lipid metabolic
Odz3	Odz, odd Oz/ten-m homolog 3	−0.56	Cell–cell signaling; signal transduction

been reported that LOU/C/Jall rats do not exhibit any deficits in synaptic plasticity up to 28 months old when compared to other rat strains (Kollen et al. 2010; Turpin et al. 2011). In keeping with the behavioral data showing intact mnemonic abilities in these rats, the present results add to these latter data, suggesting that the up-regulation of genes involved in neuronal plasticity contributes to these better abilities and thus to maintain information in short-term memory.

In the frontal cortex of LOU/C/Jall rats, a great number of genes were differentially expressed during aging. So far, only a few studies have analyzed gene expression profile in cortex during aging (Chen et al. 2004; Lu et al. 2004). When compared to the Wistar rat data set, genes were grouped into eight clearly delineated clusters. Functional profiling of these clusters revealed that genes in the frontal cortex could be involved in various biological functions. Some of them were similar to the ones already identified, such as inflammatory



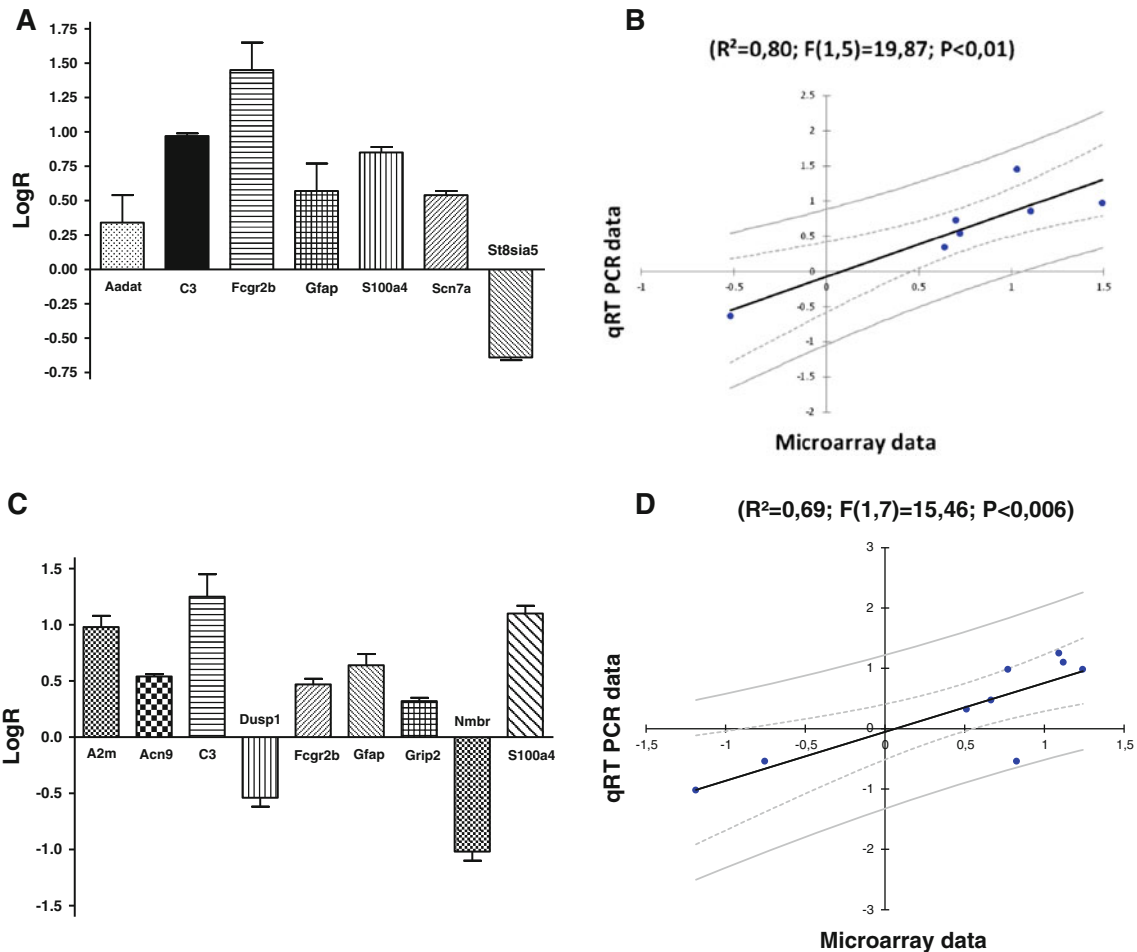


**Fig. 3** Quantitative real-time PCR validation of the microarray data for genes related to aging in hippocampus (a) and frontal cortex (c) of Wistar rats. The histograms represent the LogR ( $\pm$ SEM) of gene

expression. Regression plot between qRT PCR and microarray data for genes in hippocampus (b) and frontal cortex (d)

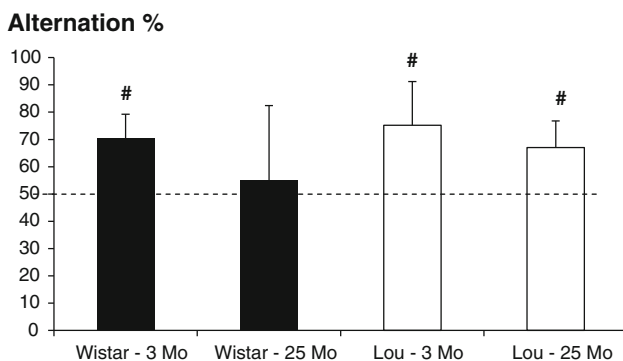
responses. It is however worth noting that there is no overlap between genes involved in this process. Of particular interest are *Acn9*, *Mosc1*, and *Mrpl10* genes. *Acn9* is a mitochondrial carrier protein associated with respiration (Steinmetz et al. 2002). *Mosc1* is involved in the mitochondrial regulation of nitric oxide biosynthesis, which in turn is a physiological mediator with versatile functions, such as the maintenance of vascular homeostasis and neuronal signaling (Kotthaus et al. 2011). *Mrpl10* belongs to the mammalian mitochondrial ribosomal proteins family (Koc et al. 2001; Yang et al. 2010). It is involved in the synthesis of protein components of the electron transport chain, as well as ATP synthase. It has been shown to be important for the regulation of the mitochondrial metabolism, cell survival, and longevity (O'Brien et al. 2005). Interestingly, these three genes were all up-regulated in the frontal cortex of LOU/C/Jall rats. One may speculate that they may contribute positively to mitochondrial functioning in aged brains, thus explaining the reduction in oxidative stress measured in LOU/C/jall rats (Garait et al. 2005). Interestingly, we identified functions associated with the homeostatic process, which is of great importance in cellular activity. For instance, *Best2* functions

as regulators of ion transport (Kunzelmann et al. 2007). Besides, *Uvrag* is involved in processes related to autophagy and apoptosis, which are crucial for cell growth and tissue homeostasis (Yin et al. 2011). This cluster of genes can be correlated with the exceptional metabolic homeostasis observed in these rats under a spontaneous caloric intake restriction (Garait et al. 2005; Veyrat-Durebex and Alliot 1997; Veyrat-Durebex et al. 1999). Another cluster of genes could be associated with the regulation of neurotransmitter levels and uptake, pointing to a high level of neuronal activity in the frontal cortex of LOU/C/Jall rats. This neuronal activity may reflect the involvement of compensatory mechanisms. Furthermore, the clusters related to the receptor metabolic process and the catabolic process including, respectively, *Grk4* (Andresen 2010) and *Unc50* (Fitzgerald et al. 2000) notably tend to reinforce the assumption that aged cortical cells in LOU/C/Jall rats still have a high level of physiological cell dynamics. This activity seemed to involve an up-regulation of biological processes within the nucleus, such as the regulation of transcription, notably through the cluster including *Aurkc* (Li et al. 2004), *Cdk12* (Bartkowiak et al. 2010) and *Klf13* (Henson and Gollin 2010) and the



**Fig. 4** Quantitative real-time PCR validation of the microarray data for genes related to aging in the hippocampus (a) and frontal cortex (c) of LOU/C/Jall rats. The histograms represent the LogR ( $\pm$ SEM)

of gene expression. Regression plot between qRT PCR and microarray data for genes in hippocampus (b) and frontal cortex (d)



**Fig. 5** Spontaneous alternation in the Y-maze. Alternation percentages (see “Materials and methods” for the formula). Data are presented as mean  $\pm$  SD. Hash indicates percentages above 50 % (univariate *t* test)

regulation of nucleus organization via the cluster including Map7 (Suzuki and Hirao 2003) and Grip2. The Grip2 protein interacts with AMPA receptors, known for being involved in

synaptic transmission and plasticity (Mao et al. 2010). Hence, as in the hippocampus, in keeping with the behavioral data, it is likely that the up-regulation of such genes related to plasticity promotes better behavioral performances. Furthermore, one may speculate that the healthier metabolic homeostasis of LOU/C/Jall rats across their lifespan, as described in detail by several authors (Alliot et al. 2002; Boghossian et al. 2002; Veyrat-Durebex and Alliot 1997; Veyrat-Durebex et al. 1999), is likely to contribute to these gene expression profiles in both the frontal cortex and the hippocampus and therefore to better cognitive abilities.

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