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Hippocampal gene expression patterns underlying the enhancement of memory by running in aged mice

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Abstract

Physical activity preserves cognition in the aging brain, but the mechanisms remain obscure. In order to identify candidate genes and pathways responsible for the preservation of cognitive function by exercise, we trained mice that had been exposed to lifelong running or sedentary lifestyle for 16 months in the hippocampus-dependent water maze. After water maze training, we analyzed the expression of 24,000 genes in the hippocampus using Illumina bead microarray. Runners show greater activation of genes associated with synaptic plasticity and mitochondrial function, and also exhibit significant downregulation of genes associated with oxidative stress and lipid metabolism. Running also modified the effects of learning on the expression of genes involved in cell excitability, energy metabolism, and insulin, MAP kinase and Wnt signaling. These results suggest that the enhancement of cognitive function by lifelong exercise is associated with an altered transcriptional profile following learning.

Keywords

Wheel running; Hippocampus; Aging; Cognition; Gene expression

1. Introduction

The aging brain retains its responsiveness to the environment. Studies have shown that environmental enrichment can influence a number of factors relevant to brain aging, including synaptic plasticity and neuronal morphology (van Praag et al., 2000). Enriched environments typically afford increased opportunities for social and cognitive stimulation, as well as physical activity. Among these components of the enrichment paradigm, physical activity in the form of wheel running has emerged as a potent modulator of central neuroplasticity. Wheel running preserves learning and memory, and enhances adult neurogenesis in the hippocampus of aging mice (van Praag et al., 2005). These effects of voluntary wheel running on learning and memory

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are associated with changes in genes expression. For example, learning (Cavallaro et al., 2002) and environmental complexity (Rampon et al., 2000) alter gene transcription in young animals. Pathways influenced by these experiences include cell-survival associated genes and genes involved in synaptic plasticity. The gene expression profile to learning has also been shown to differ between young and aged animals (Rowe et al., 2007).

The hippocampus plays a critical role in learning and memory, and is susceptible to dysfunction and degeneration in aging and Alzheimer's disease (Mattson and Magnus, 2006). Several studies have now shown that running in a wheel influences gene transcription in the hippocampus of young adult mice and rats (Molteni et al., 2002; Tong et al., 2001; Hunsberger et al., 2007). The transcripts upregulated by running overlap considerably with those influenced by enrichment and learning. In addition, the pathways influenced by running oppose the effects of aging at multiple levels. However, it remains unknown whether exercise influences the transcriptome in aging animals. Moreover, it is uncertain what changes in gene transcription might underlie the established ability of exercise to preserve learning and memory in aging animals (van Praag et al., 2005).

In these studies, we characterized the effects of lifelong running on learning-induced transcriptional modifications in aged animals. Under basal, unstimulated conditions, the differences between runners and controls were restricted to a small pool of genes. In contrast, when we compare the regulation of gene expression by learning across runners and sedentary animals, a large number of gene transcripts are differentially regulated. Running enhanced hippocampus-dependent learning in aged mice, and the enhancement of spatial memory was accompanied by differential expression of genes important for learning and synaptic plasticity, as well as genes involved in energy metabolism, and insulin and Wnt signaling. These studies suggest that aged runners differentially express certain gene transcripts in the hippocampus, and may recruit an entirely different transcriptional profile following learning.

2. Materials and methods

2.1. Animals and activity monitoring

Animal care and experimental procedures followed NIH guidelines and were approved by the National Institute on Aging Animal Care and Use Committee. Male C57BL/6 mice were housed individually with ($n = 24$) or without ($n = 18$) running wheels (Campden Instruments), beginning at 2-4 months of age. The number of wheel turns was collected automatically with custom software, and the room was maintained on a 12 h light/dark schedule with food and water available ad libitum.

2.2. Experimental design

After 16 months in their respective conditions (age at time of euthanasia = 18-20 months), runners and sedentary controls were assigned to one of the following conditions: home cage, swimmer, or learner. For analysis of basal differences in gene expression, runners ($n = 8$) and sedentary ($n = 6$) mice were rapidly sacrificed at the beginning of the light phase (8-10 a.m.), within 3 min of cage disturbance. To evaluate the regulation of gene transcription by learning, another group of runners ($n = 8$) and sedentary ($n = 6$) mice were trained in the water maze. These mice were sacrificed immediately after the probe trial on the sixth day, between 11 a.m. and 1 p.m. To control for the physiological arousal and stress associated with swimming, another group of runners ($n = 8$) and sedentary ($n = 6$) mice were allowed to swim without a target in the water maze for 7 days, before being rapidly sacrificed after the last swim session, again between 11 a.m. and 1 p.m. For tissue collection, animals were anesthetized with isoflurane, perfused with 0.9% saline, and the left hippocampi were frozen on dry ice for microarray analysis.

2.3. Water maze testing

Water maze testing took place as described (Stranahan et al., 2008). Briefly, animals received 5 days of acquisition training, consisting of four trials per day, with an intertrial interval of approximately 10 min. Each trial lasted until the animal found the platform, or for a maximum of 60 s; animals that failed to find the platform within 60 s were guided there by the experimenter. On each trial mice were placed into the pool, facing the wall, with start locations varied pseudorandomly. One day after the last acquisition training session, animals were tested in a single 60 s probe trial without the platform. Only mice that spent less than fifty percent of their time floating (defined as moving at a speed of less than 2.0 cm/s) or engaging in thigmotaxis (defined as remaining within 4.0 cm of the perimeter of the pool) were used for analysis and subsequent microarray. Data were acquired and analyzed using the HVS2020 automated tracking system (HVS Image, UK).

2.4. Serum chemistry

Glucose, 3-hydroxybutyrate, triglyceride, and cholesterol concentrations were quantified in serum using a Roche Cobas Fara II analyzer (Roche Diagnostic Systems; Montclair, NJ). Insulin concentrations in serum were determined by ELISA (Crystal Chem., Inc.). We quantified corticosterone levels in serum with radioimmunoassay using a commercially available kit (Diagnostic Products Corp., Los Angeles, CA).

2.5. Behavior and serum chemistry data analysis

Behavioral data from the water maze were analyzed using repeated measures ANOVA with Tukey's post hoc. Serum chemistry results were compared across runners and controls using *t*-tests. The amount of running over time was analyzed using Pearson's correlation. All analyses were conducted using $p < 0.05$.

2.6. RNA extraction

RNA isolation was carried out using the Qiagen RNeasy Mini Kit for animal tissues (Qiagen, Inc. Valencia CA). In short, frozen tissues were cut into small pieces and allowed to thaw in the RLT lysis buffer, on ice. This was followed by disruption using a mini-bead beater-8 and 1.0 mm glass beads (Bio-Spec Inc., Bartlesville, OK). The tissue samples were then centrifuged, the supernatant transferred to another tube, centrifuged again to clear any remaining cellular debris. The supernatant was added to 95% ethanol, mixed and added to the binding columns. The columns were centrifuged, washed several times and the bound RNA was eluted using water. The RNA quality and quantity was checked using an Agilent 2100 bio-analyzer and the RNA 6000 nano-chips.

2.7. Illumina oligonucleotide microarray

Total RNA was used to generate biotin labeled cRNA using the Illumina TotalPrep RNA Amplification Kit (Ambion; Austin, TX, cat #IL1791). In short, 0.5 µg of total RNA was first converted into single-stranded cDNA with reverse transcriptase using an oligo-dT primer containing the T7 RNA polymerase promoter site and then copied to produce double-stranded cDNA molecules. The double stranded cDNA was cleaned and concentrated with the supplied columns and used in an overnight in vitro transcription reaction where single-stranded RNA (cRNA) was generated and labeled by incorporation of biotin-16-UTP. A total of 0.75 µg of biotin-labeled cRNA was hybridized at 58 °C for 16 h to Illumina's Sentrix MouseRef-8 Expression Bead-Chips (Illumina, San Diego, CA). Each BeadChip has 24,000 well-annotated RefSeq transcripts with approximately 30-fold redundancy. The arrays were washed, blocked and the labeled cRNA was detected by staining with streptavidin-Cy3. The arrays were scanned using an Illumina BeadStation 500x Genetic Analysis Systems scanner and the image data extracted using the Illumina BeadStudio software, Version 3.0.

2.8. Microarray data analysis

Microarray data were analyzed using DIANE 6.0, a spreadsheet-based microarray analysis program based on SAS JMP7.0 system. Raw microarray data were subjected to filtering and Z normalization and tested for significant changes as described previously (Xu et al., 2007). Briefly, sample quality was analyzed by scatter plot and gene sample Z-score based hierarchical clustering to exclude possible outliers. Initial filtering identified genes with Z-ratio ≥ 1.96 , with the Z-ratio derived from the difference between the averages of the observed gene Z scores, divided by the standard deviation of all of the differences for that particular comparison. We were able to detect approximately 20,000 genes after filtering. Genes were then refined by calculating the false discovery rate (FDR), which controls for the expected proportion of falsely rejected hypotheses, and including only those genes with $FDR < 0.3$. These data were further analyzed using a 2×3 ANOVA design with significance set at $p < 0.05$. The ANOVA designed compared across physical activity (runner vs. sedentary) and environmental condition (home cage, swimming, or learning). This allowed us to identify transcripts that differed in their intensity for runners and sedentary animals. Hierarchical clustering/K-means clustering and Principal Components Analysis (PCA) was performed to identify clustering within groups. Array data for each experimental animal was also originally hierarchically clustered in Illumina BeadStudio Version 1.5.

2.9. KEGG analysis of transcriptional pathways

After identifying individual genes that were significantly regulated by different experiences in runners and sedentary mice, the gene lists were analyzed using two forms of annotational clustering, i.e. gene ontology (GO) and signaling pathway analysis (using pathways defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.genome.jp/kegg/>). We performed these two analyses on specific up- or downregulated gene sets using WebGestalt (<http://bioinfo.vanderbilt.edu/webgestalt/>) a web-based gene-set analysis toolkit. This application allows the comparison of the expression frequency of a specific gene in the experimental set to be compared to its expression frequency in the background rodent gene set that is maintained at WebGestalt. Functional clustering of genes into GO groups yields two indices that describe the degree of gene-set enrichment (R), i.e. how over-represented is this GO group in the input set compared to what one would expect from a complete background genome set and also the probability that this enrichment would occur by chance. We used a cutoff of at least two genes needing to be present to fully populate a GO term group and a probability (p) value of less than or equal to 0.05.

Similar criteria were used for the clustering of genes into functional signaling pathways (derived from KEGG). Classification of significantly populated KEGG groups is considerably less likely than into GO term groups as the number of potential gene identities that can fit into any one KEGG pathway is relatively small compared to the number of GO terms that can be applied to a single gene. Hence in the majority of cases more significantly populated GO term groups were observed for each input gene set than KEGG pathways. Both modes of annotation attribute a phenotypic description of the output gene set.

3. Results

3.1. Running enhances hippocampus-dependent learning in aged mice

All mice housed with running wheels ran extensively (mean distance = 1.54 ± 0.32 km/day) and there was a significant decrease in running activity with age (Pearson's correlation, time $\times$ distance; $r^2 = 0.76$, $p = 0.0005$). In concurrence with previous reports (van Praag et al., 2005) running was associated with reduced escape latency in the maze ($F_{1,48} = 6.81$, $p = 0.02$; Fig. 1A). Runners also took a more direct path to the platform ($F_{1,48} = 5.76$, $p = 0.003$; Fig. 1B). In the probe trial, animals that had engaged in lifelong running spent more time searching in

the target quadrant ($F_{3,21} = 3.91$, $p = 0.02$; Fig. 1C). There were no differences in swim speed between runners and sedentary animals ($F_{1,48} = 0.68$, $p = 0.42$ cm/s, sedentary = 13.80 ± 0.49 , runner = 13.13 ± 0.48).

We observed no difference between runners and sedentary mice in performance on the visible platform version of the water maze, which is not hippocampus-dependent ($t_{12} = 0.02$, $p = 0.98$; mean latency across four trials, sedentary = 23.21 ± 3.73 , runner = 23.34 ± 5.76). These results support a role for physical activity in preserving hippocampal function in aging animals, but it is uncertain what molecular pathways might mediate these effects.

3.2. Serum chemistry alterations with lifelong running

Runners had reduced circulating insulin concentrations in the postprandial state ($t_{11} = 4.93$, $p = 0.0004$; ng/ml, control = 2.27 ± 0.21 ; runner = 1.02 ± 0.14). There was no change in fed serum glucose levels ($t_{40} = 0.06$, $p = 0.95$; mg/dL, control = 157.8 ± 8.53 , runner = 158.4 ± 6.61). However, we did observe an increase in 3-hydroxybutyrate concentrations with lifelong running ($t_{12} = 2.56$, $p = 0.03$; mg/dL, control = 0.52 ± 0.08 , runner = 0.88 ± 0.11). Overall, these results are suggestive of improved insulin sensitivity and ketone metabolism in aged runners, without any indication of pathological changes in glucose and insulin levels in aged sedentary mice.

Cholesterol levels were similar across runners and controls ($t_{40} = 0.78$, $p = 0.44$; mg/dL, control = 119.1 ± 6.19 , runner = 114.30 ± 2.65); however, runners had significantly lower levels of low-density lipoprotein ($t_{39} = 2.48$, $p = 0.02$; mg/dL, control = 52.58 ± 3.71 , runner = 43.02 ± 1.91), with commensurate alterations in the HDL:LDL ratio ($t_{39} = 2.45$, $p = 0.02$; HDL/LDL ratio, controls = 1.40 ± 0.09 , runner = 1.73 ± 0.08). Levels of serum triglycerides were not altered by lifelong running ($t_{40} = 0.14$, $p = 0.88$; mg/dL, control = 126.5 ± 6.98 , runner = 128.2 ± 9.49). These results suggest that running improves the serum lipid profile of aged mice, but there is no indication of pathology in aged sedentary mice.

Serum corticosterone concentrations were also similar across runners and controls (Supplementary Figure 1). There were no differences in basal corticosterone concentrations following 6 days of swimming without a target in the water maze, or learning to locate the hidden platform. Moreover, there was no significant effect of learning or swimming relative to mice sacrificed directly out of the home cage, suggesting that at the time of euthanasia, the stress status of mice in all three conditions was similar.

3.3. Genes and pathways regulated by learning

3.3.1. Different genes are regulated by learning in runners and sedentary mice

—There were no significant differences in the number of transcripts that met our filtering criteria across runners and sedentary mice ($t_{40} = 0.38$, $p = 0.69$; sedentary = 19.990 ± 146.0 , runner = 19.920 ± 91.72). The number of transcripts regulated by learning was also similar across runners and controls (Fig. 2). In sedentary animals, 169 transcripts met our criteria and were expressed only in sedentary mice following water maze training. Of those 169 transcripts, most genes (~65%) were upregulated. Transcripts upregulated by learning in sedentary mice included transcription factors (Zfml [GeneID: 18139], Foxg1 [GeneID: 15228], Rnf187 [GeneID: 108660]), as well as genes associated with potassium channel function (Kcnp4 [GeneID: 80334], Kctd12 [GeneID: 239217]; Supplementary Table 1A). Transcripts downregulated by learning in sedentary mice included genes implicated in the oxidative stress response (Gpx1 [GeneID: 14775]), and mitochondrial function (Bag1 [GeneID: 12017]; Supplementary Table 1B).

In runners, 176 transcripts met our criteria and were expressed exclusively in runners following learning (Fig. 2). Of those 176 genes, the majority (~72%) were upregulated. Genes upregulated by learning in runners included a distinct pool of transcription factors not regulated by learning in sedentary animals including *Egr4* [GeneID: 13656]. Other upregulated transcripts encode genes essential for noradrenergic neurotransmission (*Adrbk1* [GeneID: 110355]), as well as transcripts implicated in microtubule dynamics (*Map1lc3a* [GeneID: 66734], *Eml1* [GeneID: 68519]; Supplementary Table 2A). Transcripts downregulated by learning in runners included genes associated with neuronal calcium metabolism (*HpcA* [GeneID: 15444], *Cacna1h* [GeneID: 58226], *Camk2a* [GeneID: 12322]; Supplementary Table 2B). However, based on the level of dispersion in our principal component analysis (Supplementary Figure 2), it is apparent that alterations in a small number of genes cannot account for the enhancement of hippocampal function in aged runners.

3.3.2. Different pathways are influenced by learning in runners and sedentary mice—Analysis using the KEGG database revealed that 13 pathways were significantly altered by learning in sedentary mice (Fig. 3). Of these pathways, ~15% were upregulated (Supplementary Table 1C) and ~85% were downregulated (Supplementary Table 1D). Ribosomal signaling was bidirectionally influenced by learning in sedentary mice, with separate sets of transcripts implicated in ribosomal function significantly up- or downregulated. Learning also increased oxidative phosphorylation in sedentary mice, and reduced expression of genes in the Wnt, hedgehog, and mitogen-activated protein kinase signaling pathways.

Runners showed alterations in the expression of genes in 21 pathways following training in the water maze (Fig. 3). Of these pathways, ~29% were upregulated (Supplementary Table 2C) and ~71% were downregulated (Supplementary Table 2D). Transcripts in the Wnt signaling pathway were detected in both the up- and downregulated pathways. The expression of genes in pathways implicated in fructose, mannose, and butanoate metabolism were changed following learning in runners, while pathways involved in long-term potentiation and long-term depression were reduced. Quantitatively, this suggests that runners show changes in gene transcription across more diverse pathways than sedentary animals following learning. Qualitatively, the pathway analysis suggests that enhanced cerebral metabolism may underlie the improved performance of runners in the water maze.

We also performed an additional analysis of the various cellular processes that were influenced by learning in runners and sedentary mice. To do this, we used the KEGG database to extract the Gene Ontology (GO) terms associated with different categories of transcripts. Analysis of GO terms altered by learning in sedentary mice identified 30 functional categories, the majority of which were upregulated (60%; Supplementary Table 1E-F). Upregulated categories included genes involved in the regulation of cellular morphology, axon guidance, and protein synthesis (Supplementary Figure 3). Downregulated categories included transcripts involved in calmodulin binding and mitochondrial function (Supplementary Figure 4).

In runners, the number of cellular processes regulated by learning was greater than in sedentary mice; 44 GO terms were significantly influenced by learning. Among these 44, more than half were upregulated (~56%; Supplementary Table 2E-F). Categories of upregulated transcripts included genes associated with dendritic spines and memory (Supplementary Figure 3). Because running enhances both dendritic spine density (Stranahan et al., 2007) and learning (van Praag et al., 1999), this raises the possibility of a link between these two categories. Downregulated categories include genes associated with oxidative phosphorylation and ATP synthesis (Supplementary Figure 4), indicating that runners may respond more efficiently to the energetic demands associated with learning and memory.

3.3.3. Transcripts commonly regulated by learning in both runners and sedentary mice

—To identify transcripts regulated by learning in both runners and sedentary mice, we removed all transcripts that were influenced by swimming, and transcripts that differed between runners and sedentary mice under baseline, home cage conditions. There were 78 genes that were expressed in the hippocampus of both runners and sedentary animals following water maze training (Fig. 2). Of these 78, most (~68%) showed increased signal intensity. Transcripts implicated in synaptic plasticity (Syngr3 [GeneID: 20974], Scg2 [GeneID: 20254]) and mitochondrial function (Cyp2j9 [GeneID: 74519], Tomm7 [GeneID: 66169], Cyb5 [GeneID: 109672], Bag4 [GeneID: 67384]) were increased by learning in both runners and sedentary animals (Supplementary Table 3A). Other genes implicated in excitatory synaptic transmission were reduced (Grina [GeneID: 66168], Syt1 [GeneID: 20979]; Supplementary Table 3B). This is consistent with previous studies of learning-induced transcriptional alterations in aged rats (Rowe et al., 2007).

KEGG analysis revealed that 12 pathways were commonly regulated by learning in both runners and sedentary mice (Fig. 3). Within these 12, ~17% were upregulated (Supplementary Table 3C) and ~83% were downregulated (Supplementary Table 3D). Transcripts associated with purine and pyrimidine metabolism were upregulated, while genes associated with long-term potentiation and calcium signaling were among the downregulated pathways. Other significantly downregulated pathways included the Wnt signaling pathway and genes involved in apoptosis. These findings suggest that learning places a higher metabolic demand on the hippocampus, and suppresses apoptotic gene transcription in aged mice.

Extraction of GO terms identified 20 functional categories of transcripts regulated by learning in runners and sedentary mice (Supplementary Table 3E-F). Among these categories, a few (~35%) were upregulated, but most were downregulated (~65%). Genes implicated in cytochrome *c* activation and metabolism of reactive oxygen species were downregulated in runners and sedentary mice following learning, suggesting that mental activity may be protective in the aging brain (Supplementary Figure 10). Additional transcripts associated with the endoplasmic reticulum and oxidoreductase activity were among the upregulated categories; reinforcing the possibility that mental activity may induce a hormetic stress response (Supplementary Figure 9; Arumugam et al., 2006).

3.4. Genes and pathways regulated by swimming

3.4.1. Different genes are regulated by swimming in runners and sedentary mice

—We also examined genes that were regulated by swimming, but not by learning, in runners and sedentary mice. Sedentary mice expressed 95 transcripts that were significantly up- or downregulated following swimming without a target in the water maze (Fig. 2). Within this set, a minority showed reduced signal intensity (~25%), while the majority of genes (~75%) were downregulated. Downregulated genes included transcripts that are involved in GABAergic neurotransmission (Gabra1 [GeneID: 57436], Gabrb1 [GeneID: 14400]) and chloride channel function (Clcn3 [GeneID: 12725]; Supplementary Table 4B). Upregulated genes included transcripts implicated in the stress response (Hspb8 [GeneID: 80888]), as well as genes involved in ketone metabolism (Hibadh [GeneID: 58875]; Supplementary Table 4A).

Runners recruited a larger pool of transcripts following swimming than sedentary animals. There were 166 genes that were specific to swimming in runners. Of these transcripts, ~30% were upregulated and ~70% were downregulated (Fig. 2). In runners, genes involved in energy metabolism (Atp5h [GeneID: 71679], Atp6v1c1 [GeneID: 66335]) and the stress response (Crhbp [GeneID: 12919]) were upregulated following swimming (Supplementary Table 5A), while genes involved in glutamatergic neurotransmission (Slc1a1 [GeneID: 20510]) and synaptic structure were downregulated (Cnnt2 [GeneID: 21367], Syn2 [GeneID: 20965],

Dctn2 [GeneID: 69654]; Supplementary Table 5B). This indicates that runners respond to swimming with qualitatively different alterations in genes involved in synaptic transmission.

3.4.2. Different pathways are influenced by swimming in runners and sedentary mice—Analysis of transcripts using the KEGG database identified four pathways that were responsive to swimming in sedentary mice. Within these four categories, one functional grouping was upregulated, and the rest were downregulated. Transcripts involved in long-term depression and gap junctional communication were downregulated (Supplementary Table 4D), while transcripts implicated in amino acid metabolism were upregulated (Supplementary Table 4C).

Compared to sedentary mice, runners exhibited changes in the expression of genes in a larger set of pathways following swimming. Using the KEGG database, we identified 15 categories that were responsive to swimming in runners. Among these 15, two categories were upregulated, while most (13/15) were downregulated. We observed reduced signal intensity for transcripts involved in insulin signaling and the mammalian target of rapamycin signaling pathway (Supplementary Table 5D). At the same time, we observed increased signal intensity for transcripts expressed in ribosomes, and for transcripts involved in oxidative phosphorylation (Supplementary Table 5C).

We also extracted the GO terms associated with transcripts that were regulated by swimming in runners and sedentary mice. Sedentary mice expressed transcripts associated with 22 different GO terms following swimming. Within these 22 GO terms, a few (6/22) were upregulated, while majority were downregulated (Supplementary Table 4E-F). Genes involved in neurotransmitter release and synaptic structure were downregulated (Supplementary Figure 5), while genes implicated in fatty acid metabolism and mitochondrial function were upregulated (Supplementary Figure 5).

In runners, we identified 23 GO terms that were responsive to swimming (Supplementary Table 5E-F). Again, fewer GO terms were upregulated, as most GO terms (16/23) were derived from downregulated transcripts. Transcripts involved in neurotransmitter metabolism and microtubule structure showed reduced signal intensity (Supplementary Figure 6), while genes associated with potassium channel activity and ATP synthesis were increased (Supplementary Figure 5).

3.4.3. Shared genes responsive to swimming in runners and sedentary mice—

We identified 20 genes that were expressed following swimming in both runners and sedentary animals (Fig. 2). Fewer genes were upregulated (25%), as most transcripts showed reduced signal intensity (75%). Upregulated genes included transcripts involved in cytoskeletal regulation (Nrn1 [GeneID: 68404]) and chromatin structure (H2afz [GeneID: 51788]; Supplementary Table 6A), and downregulated genes include transcripts implicated in lipid metabolism (Ogdh [GeneID: 18293]) and astroglial function (Pea15 [GeneID: 18611]; Supplementary Table 6B). Because the number of genes activated following swimming in runners and sedentary mice is relatively small, we did not detect any significant alterations at the level of pathway analysis using the KEGG database. However, extraction of GO terms revealed that 13 categories of transcripts were influenced by swimming in runners and sedentary mice (Supplementary Table 6C-D). Approximately 46% of GO terms were upregulated following swimming. Transcripts expressed in the cytosol and intracellular organelles were upregulated (Supplementary Figures 5 and 9), while genes implicated in regulation of cellular morphology and cell-cell adhesion were downregulated (Supplementary Figures 6 and 10). These results are suggestive of compartmentalized responses to swimming in runners and sedentary mice.

3.5. Transcripts and pathways regulated by learning and swimming

3.5.1. Transcripts common to learning and swimming—When we look exclusively at sedentary mice, there were 37 transcripts that were common to learning and swimming (Fig. 4). When we examined the transcriptional profile in runners, there were 83 transcripts common to learning and swimming that were not expressed in sedentary mice. This suggests that physically active mice recruit more common transcripts following learning and swimming. There were also 90 genes that were common to learning and swimming in both runners and sedentary mice.

Within the 37 transcripts regulated by swimming in sedentary mice, approximately half (~54%) were upregulated (Fig. 4). Upregulated genes include transcription factors (Phf5a [GeneID: 68479]) and genes involved in cell cycle progression (Skp1a [GeneID: 21402]; Supplementary Table 7A). Among the downregulated genes, we observed significant reductions in transcripts important for mitochondrial function (Uqcrc1 [GeneID: 22273], Atp5d [GeneID: 66043]) and proteasomal degradation (Psm4 [GeneID: 19185]; Supplementary Table 7B). Among the 83 transcripts influenced by swimming in runners, approximately half (~48%) were increased (Supplementary Table 8A-B). We observed increased signal intensity for transcripts implicated in synaptic transmission (Slc35b4 [GeneID: 58246], Slc25a14 [GeneID: 20523], Viat [GeneID: 22348]) and energy metabolism (Mrpl53 [GeneID: 68499], Ndufs2 [GeneID: 226646], Mrps12 [GeneID: 24030]), suggesting that runners may be co-recruiting transcriptional mechanisms for learning and swimming.

There were 90 genes expressed following learning and swimming in both runners and sedentary mice (Fig. 4). Within this set, the majority (~63%) were upregulated. Signal intensities for transcripts important for glutamatergic neurotransmission (Calm2 [GeneID: 12314], Glul [GeneID: 14645]) and ribosomal function were increased (Rpl18a [GeneID: 76808], Rps6 [GeneID: 20104]; Supplementary Table 9A), while signal intensities for other transcripts involved in glutamatergic neurotransmission were decreased (Grip1 [GeneID: 74053]; Supplementary Table 9B). Quantitatively, this pattern suggests that runners recruit more common genes following learning and swimming. These data also suggest that there is a high degree of overlap between runners and sedentary animals with respect to the transcriptional response to learning and swimming.

3.5.2. Pathways activated by both learning and swimming—Analysis of transcripts using the KEGG database revealed that 11 categories were commonly activated following learning and swimming in runners and sedentary mice (Fig. 4). Pathways that were enhanced following learning and swimming include the mitogen-activated protein kinase signaling cascade and the insulin signaling pathway (Supplementary Table 9C); pathways with reduced activation included transcripts implicated in glycerophospholipid metabolism (Supplementary Table 9D). The majority of pathways (10 out of 11) were upregulated.

We also identified 2 pathways that were affected by both learning and swimming in sedentary mice, but not in runners (Fig. 4). The upregulated category included transcripts involved in cell cycle regulation (Supplementary Table 7C), while the downregulated category was comprised of transcripts implicated in oxidative phosphorylation (Supplementary Table 7D). Runners, in contrast, exhibit changes in 6 common pathways following learning and swimming; all 6 pathways were downregulated (Supplementary Table 8C). These shared pathways include transcripts important for long-term potentiation and calcium signaling.

Analysis at the level of GO terms allowed us to characterize additional cellular processes and anatomical compartments influenced by learning and swimming in runners and sedentary mice. There were 24 GO terms influenced by learning and swimming in both runners and sedentary mice. Within these 24, most (18/24) were upregulated. Transcripts associated with

mitochondrial function and cytoskeletal organization were upregulated (Supplementary Figure 9; Supplementary Table 9E), while other transcripts associated with axonogenesis were downregulated (Supplementary Figure 10; Supplementary Table 9F).

When we looked at GO terms influenced by learning and swimming exclusively in sedentary mice, we found 11 categories that were responsive to both experiences. Among these eleven, 5 were upregulated (Supplementary Table 7E) and 6 were downregulated (Supplementary Table 7F). Genes involved in RNA processing and protein transport was upregulated (Supplementary Figure 7); downregulated transcripts included genes associated with mitochondrial function and energy metabolism (Supplementary Figure 8).

In runners, we identified 20 GO terms commonly regulated by learning and swimming (Supplementary Table 8D-E). Of these 20, 13 were upregulated, and 7 were downregulated. Upregulated GO terms included the mitochondrial compartment and the cytoskeleton (Supplementary Figure 7). Transcripts that fell under downregulated GO terms included genes involved in learning and synaptogenesis (Supplementary Figure 8).

3.6. Differences in expression levels for transcripts and pathways following learning, swimming, or under home cage conditions

3.6.1. Differences in expression levels between runners and controls after learning—In addition to observing recruitment of distinct groups of transcripts following learning, we also observed differences in expression levels within the same genes across runners and sedentary mice. That is to say that while some genes were expressed in runners and not in sedentary mice following specific experiences, other genes were expressed in both runners and sedentary mice at different levels. Following 6 days of training in the water maze, there were 391 transcripts that were differentially expressed in runners, relative to sedentary mice (Fig. 5). Of those 391, a smaller proportion of transcripts showed increased signal intensity, as the majority (~61%) were downregulated in runners. Transcripts implicated in the mitogen-activated protein kinase cascade (Mapk8ip [GeneID: 19099]) and potassium channel function (Kcna6 [GeneID: 16494], Hcn2 [GeneID: 15166]) were prominent among the upregulated genes (Supplementary Table 10A), while genes involved in proteasomal degradation (Psmbl1 [GeneID: 19170], Senp3 [GeneID: 80886], Psme3 [GeneID: 19192]) and mitochondrial function (Mrps18c [GeneID: 68735], Timm8b [GeneID: 30057], Idh2 [GeneID: 269951]) were present among the downregulated genes (Supplementary Table 10B). The global pattern of changes in the relative signal intensities for different transcripts are suggestive of enhanced synaptic plasticity in aged runners.

KEGG analysis of pathways altered following learning revealed that 34 transcriptional cascades were differentially regulated between runners and controls. Specifically, this suggests that among the same set of pathways, runners and controls may show different levels of activation. Among these pathways, ~38% were upregulated (Supplementary Table 10C) and ~62% were downregulated (Supplementary Table 10D). Pathways that were differentially upregulated included the mitogen-activated protein kinase cascade, and transcripts associated with inositol phosphate metabolism. Pathways that were differentially downregulated included genes essential for ribosomal function, as well as focal adhesion molecules and regulators of the actin cytoskeleton.

Extraction of GO identified 59 cellular processes and compartments that were differentially activated between runners and sedentary mice after learning (Supplementary Table 10E-F). Among these 59, 34 were upregulated (Supplementary Figure 11), and 25 were downregulated (Supplementary Figure 12). Upregulated GO terms included acetylcholine receptor signaling and learning; downregulated GO terms included synapse organization and ATP synthesis.

3.6.2. Differences in gene expression levels between runners and controls after swimming—

We also evaluated the relative expression levels of different transcripts after swimming in runners and sedentary mice (Fig. 5). There were 167 genes that were differentially expressed following swimming; of these genes, ~62% were upregulated (Supplementary Table 11A), and 38% were downregulated (Supplementary Table 11B). It is of interest to note that differences in gene expression between runners and controls following learning showed greater transcriptional downregulation, while differences following swimming were primarily attributable to upregulation. Several protein chaperones (Cct3 [GeneID: 12462], Cct4 [GeneID: 12464], Hspcb [GeneID: 15516], Cct6a [GeneID: 12466]) were upregulated in runners relative to sedentary animals after swimming. Transcripts involved in the mitogen-activated protein kinase signaling cascade (Map2k2 [GeneID: 26396]) were downregulated in runners after swimming.

KEGG analysis identified 8 pathways that were differentially affected by swimming in runners and sedentary mice (Supplementary Table 11C-D). Among these pathways, a small minority was derived from upregulated transcripts, as most (7 out of 8) were downregulated. The fructose and mannose metabolic pathway was differentially activated in runners and sedentary mice following swimming. The mitogen-activated protein kinase pathway was among the pathways with reduced activation, as were pathways involved in gap junctional communication and neurodegenerative disease.

Extraction of GO terms revealed 31 functional categories of transcripts that were differentially influenced by swimming in runners and sedentary mice (Supplementary Table 11E-F). There were fewer GO terms derived from the upregulated genes (13/31), relative to the downregulated transcripts. Genes implicated in epigenetic modulation of chromatin structure and transcripts involved in ATP production were among the upregulated categories (Supplementary Figure 11), while transcripts involved in neurogenesis and potassium channel function were downregulated (Supplementary Figure 12). The overall pattern suggests that the transcriptional profile following swimming differs between runners and sedentary mice.

3.6.3. Baseline differences in gene expression levels in aged runners and sedentary mice—

We detected 42 transcripts that were differentially expressed in runners under baseline, home cage conditions. Of these 42 transcripts, a few showed increased signal intensity (~31%), while most (~69%) were downregulated (Fig. 5). A number of transcripts important for oxidative stress (Gpx1 [GeneID: 14775], Uqcrc1 [GeneID: 22273]) and mitochondrial function (Atp5d [GeneID: 66043]) were downregulated in aged runners (Supplementary Table 12B). We also observed significant upregulation of genes associated with stress responses (Hsp105 [GeneID: 15505]) and glial modulation of glutamatergic neurotransmission (Slc1a3 [GeneID: 20512]; Supplementary Table 12A).

Because the number of genes that differ in their expression between runners and controls under home cage conditions was relatively small, we were not able to detect any changes at the level of KEGG pathway analysis. However, extraction of GO terms allowed us to identify 11 functional categories of genes that differed between runners and sedentary mice under home cage conditions (Supplementary Table 12C-D). Most of these functional categories (8 out of 11) were downregulated; the downregulated categories included apoptotic genes, and transcripts implicated in mRNA processing (Supplementary Figure 12). Upregulated categories included transcripts associated with ribosomes, and genes involved in protein trafficking (Supplementary Figure 11). This pattern suggests that runners may experience reduced apoptosis, and more efficient transcription and translation of proteins under home cage conditions. Quantitatively, however, these results suggest that more differences in hippocampal gene transcription in aged runners occur following cognitive or physiological challenges.

4. Discussion

Running enhances hippocampus-dependent learning in aged mice. Improvements in learning were not attributable to changes in a small number of genes, but instead involved changes in the number and diversity of the molecular pathways influenced by water maze training. Runners recruited a more varied set of transcriptional categories following learning. These categories included transcripts involved in dendritic and synaptic plasticity, as well as genes implicated in learning and memory. Mice exposed to lifelong running also showed greater activation of metabolic pathways in the hippocampus following maze training, suggesting that they are better able to meet the energetic demands associated with learning.

Running modified the effects of learning on the expression of genes involved in insulin-like signaling, a pathway believed to play important roles in aging (Rincon et al., 2005; Reagan, 2007), regulation of brain energy metabolism (Hoyer and Lannert, 2007), neurogenesis (Aberg et al., 2000) and synaptic plasticity and learning and memory (Biessels et al., 1998; Benedict et al., 2004; Stranahan et al., in press). The mechanism by which exercise and learning modify insulin signaling in brain cells is unknown, but could involve effects on neuronal activity and energy metabolism, or could be secondary to peripheral effects on glucose metabolism. MAP kinase and Wnt signaling are two other pathways that we found were modified by learning and running. MAP kinases are well known for their roles in neurotrophic factor signaling (Reichardt, 2006) and synaptic plasticity (Sweatt, 2004), and so are poised to be important mediators of the effects of environmental challenges on neuronal structure and function. Wnt signaling is also involved in processes associated with hippocampal plasticity and learning and memory including neurogenesis (Lie et al., 2005) and LTP (Chen et al., 2006). The roles of Wnt signaling in exercise-induced hippocampal plasticity and learning and memory remain to be established.

We also observed that mice exposed to lifelong running exhibit greater overlap between learning and swimming, with regard to the transcriptional response among cells in the hippocampus. Runners co-recruit more genes following learning and swimming, and also regulate a more diverse set of molecular pathways. This suggests that the enhancement of learning in runners may arise from co-recruitment of mechanisms involved in the response to swimming. We did not observe many differences in hippocampal gene expression between runners and sedentary mice under basal, home cage conditions. However, our animals were sacrificed during the light phase, when very little running occurs. It is possible that differences in gene transcription might have been evident if the animals had been euthanized at the time of their highest activity levels in the wheel. Additionally, it is likely that long-term voluntary running leads to changes at other levels, such as translational efficiency or protein stability, that may replace early transcriptional responses to exercise.

In the current study, we used lifelong running to investigate the effects of physical activity on cognition in the aging brain. Other studies have assessed the effects of late-onset exercise, with mixed results. While van Praag et al. (2005) observed that 45 days of running enhanced water maze learning in 19-month-old mice, Nichol et al. (2007) reported that 21 days of running had no effect on the performance of aged mice in a water escape motivated radial arm maze. This could reflect differences in the cognitive demands imposed by the two tasks, or a threshold effect occurring between 21 and 45 days of running. However, taken together with our data, the findings of van Praag et al. (2005) suggest that at least in the water maze, both lifelong and late-onset running may have beneficial effects on cognition.

Throughout the manuscript, we refer to ‘learning’ in the broadest sense, in that associative learning requires the acquisition, encoding, storage, and recall of information. However, because we measured gene transcription directly after the probe trial, our results most likely

reflect the molecular mechanisms underlying memory recall. It remains to be seen whether similar mechanisms are acting during earlier phases of acquisition, encoding, and storage of newly acquired memories.

Wheel running enhances synaptic plasticity, dendritic spine density, and adult neurogenesis in the hippocampus (van Praag et al., 1999; Stranahan et al., 2006; Stranahan et al., 2007). Many of these processes share common mechanisms; for example, excitatory synaptic input is critical for synaptic plasticity, spinogenesis, and adult neurogenesis (Malenka and Bear, 2004; Sorra and Harris, 2000; Nacher and McEwen, 2006). Because we have observed alterations in a number of transcriptional pathways implicated in plasticity, it is possible that the specific transcripts altered in aged runners may account for the enhancement of learning and memory. Higher levels of physical activity are also correlated with successful aging in human populations (Hillman et al., 2008), opening the possibility that the mechanisms expressed following learning in aged runners may also protect against age-related memory loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Lifelong running enhances hippocampus-dependent memory in aged mice. (A) Latency to find the hidden platform in the water maze was lower in mice with lifelong access to a running wheel. (B) Runners also took a more direct route to the platform. (C) During the first 10 s of the probe trial, runners spent significantly more time in the target quadrant than sedentary mice. Asterisk (*) indicates significance at $p < 0.05$ following one-way repeated measures ANOVA.

Fig. 2.

Lifelong running alters the hippocampal transcriptome following learning or swimming. (A) Heat map plot showing patterns of gene transcription in sedentary mice or runners following swimming without a target, or learning in the water maze. (B) Similar numbers of transcripts are altered by learning in sedentary mice and runners. (C) Runners (RUN) and sedentary mice (SED) show parallel alterations in up- or downregulation of gene transcription following learning. A small pool of common genes induced by water maze training (COM) also follows this pattern. (D) Runners recruit a greater transcriptional response following swimming without a target, relative to sedentary mice. (E) Despite differences in the number of transcripts regulated by swimming, runners and sedentary mice show similar proportions of up- and downregulated genes. For a complete list of transcripts activated by learning exclusively in sedentary mice, see Supplementary Table 1A-B; for a complete list of transcripts activated by learning exclusively in runners, see Supplementary Table 2A-B; for a list of transcripts

responsive to learning in both runners and sedentary mice, see Supplementary Table 3A-B. To view transcripts activated by swimming in sedentary mice, see Supplementary Table 4A-B; for transcripts induced by swimming in runners, see Supplementary Table 5A-B; for a list of transcripts responsive to swimming in both runners and sedentary mice, see Supplementary Table 6A-B.

Fig. 3.

Transcriptional pathways activated by learning in the hippocampus of runners and sedentary mice. (A) Numbers of pathways that were responsive to learning in runners (RUN) and sedentary mice (SED). (B) Direction of changes in expression of different transcriptional pathways following learning. In this context, common (COM) refers to pathways regulated by learning in both runners and sedentary mice. (C) Transcripts associated with ribosomes were differentially regulated following learning in runners and sedentary mice. (D) The Wnt signaling pathway was differentially regulated following learning in runners and sedentary mice. For a complete list of pathways regulated by learning in runners, see Supplementary Table 1C-D; for sedentary mice, see Supplementary Table 2C-D; for a list of pathways responsive to learning in both runners and sedentary mice, see Supplementary Table 3C-D.

Fig. 4.

Co-regulation of transcripts and pathways following learning and swimming in runners and sedentary mice. (A) Venn diagram showing the number of transcripts induced following both learning and swimming in sedentary mice (SED) and runners (RUN). (B) Runners recruit more shared transcripts following learning and swimming than sedentary mice. (C) Pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database revealed that runners recruit a more diverse set of pathways than sedentary mice following learning and swimming. In this context, common (COM) refers to genes or pathways that are commonly regulated by learning and swimming in both runners and sedentary mice. For a complete list of transcripts

co-regulated by learning and swimming in sedentary mice, see Supplementary Table 7A-B; for runners, see Supplementary Table 8A-B; for both, see Supplementary Table 9A-B.

Fig. 5.

Differences in expression levels within the same pool of transcripts under home cage conditions, or following learning or swimming. (A) Heat map showing the transcriptional profile under home cage conditions, following swimming without a target, or learning in the water maze. (B) The signal intensity for a number of transcripts was altered by running following different experiences. (C) Differences in signal intensity across runners and sedentary mice were primarily attributable to downregulation of transcripts following learning; in contrast, differences following swimming were mainly due to upregulation. A small number of transcripts were differentially expressed under home cage conditions; this effect was comprised mainly of downregulated transcripts. For a complete list of transcripts expressed at

different levels in runners and sedentary mice following learning, see Supplementary Table 10A-B; for a complete list of transcripts expressed at different levels in runners and sedentary mice following swimming, see Supplementary Table 11A-B; for a complete list of transcripts expressed at different levels in runners and sedentary mice under home cage conditions, see Supplementary Table 12A-B.