

THE EVOLUTION OF GENE EXPRESSION IN MOUSE HIPPOCAMPUS IN RESPONSE TO SELECTIVE BREEDING FOR INCREASED LOCOMOTOR ACTIVITY

A. M. BRONIKOWSKI,¹ J. S. RHODES,² T. GARLAND, JR.,³ T. A. PROLLA,⁴ T. A. AWAD,⁵ AND S. C. GAMMIE⁶

¹Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011

E-mail: abroniko@iastate.edu

²Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, Oregon 97239

E-mail: rhodesju@ohsu.edu

³Department of Biology, University of California, Riverside, California 92521

E-mail: tgarland@citrus.ucr.edu

⁴Department of Genetics and Medical Genetics, University of Wisconsin, Madison, Wisconsin 53706

E-mail: taprolla@facstaff.wisc.edu

⁵Affymetrix Corporation, 3380 Central Expressway, Santa Clara, California 95051

E-mail: tarif_awad@affymetrix.com

⁶Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

E-mail: scgammie@facstaff.wisc.edu

Abstract.—The evolution of behavior has been notoriously difficult to study at the molecular level, but mouse genetic technology offers new promise. We applied selective breeding to increase voluntary wheel running in four replicate lines of *Mus domesticus* (S mice) while maintaining four additional lines through random breeding to serve as controls (C mice). The goal of the study was to identify the gene expression profile of the hippocampus that may have evolved to facilitate the increased voluntary running. The hippocampus was of interest because it is known to display marked physiological responses in association with wheel running itself. We used high-density oligonucleotide arrays representing 11,904 genes. To control for the confounding influence of physical activity itself on gene expression, animals were housed individually without access to running wheels, and were sampled during the day when they are normally inactive. Two-month-old female mice in estrus were used ($n = 16$ total; two per line; 8 S and 8 C). After correcting for an acceptable false discovery rate (10%), 30 genes, primarily involved in transcription and translation, significantly increased expression whereas 23 genes, distributed among many categories including immune function and neuronal signaling, decreased expression in S versus C mice. These changes were relatively small in magnitude relative to the changes in gene expression that occur in the hippocampus in response to wheel running itself. A priori tests of dopamine receptor expression levels demonstrated an increase of approximately 20% in the expression of D2 and D4 receptors. These results suggest that relatively small changes in the expression patterns of hippocampal genes underlie large changes in phenotypic response to selection, and that the genetic architecture of running motivation likely involves the dopaminergic system as well as CNS signaling machinery.

Key words.—Artificial selection, dopamine, exercise, microarray, transcription.

Received February 12, 2004. Accepted May 30, 2004.

The evolution of behavior has been notoriously difficult to study at the molecular level, that is, to pinpoint the loci at which changes in allele frequency correspond to changes in the behavioral phenotype. This is due to the complexity of behavioral traits, including polygenic inheritance, and the lability of many behaviors such that the behavioral “trait” can be difficult to define distinctly from its plasticity. For behaviors for which these obstacles can be overcome, mapping the genotype-to-phenotype relationship has depended on the a priori identification of candidate genes of relatively small numbers and large effects. As Anholt et al. (2003) have recently argued, behavioral traits are at the “apex of biological complexity” because many genes generally affect them whose expression may depend on the expression of other genes in their regulatory networks. With the advent of high-throughput methods for surveying the expression of thousands of genes simultaneously, we have the tools to understand not only the molecular genetic basis of complex behavioral traits (e.g., Rampon et al. 2000), but to identify the changes in gene expression that are associated with behavioral evolution.

Traditionally, the search for genes important in the evolution of behavior has depended on the identification of candidate genes through mutant screens (e.g., Bartke et al. 2001),

and screens for quantitative trait loci (e.g., Peripato et al. 2002). For example, mice homozygous for a mutation in the PROP-1 gene have a deficient primary pituitary gland that results in a lack of growth and thyroid hormones. Behavioral and learning screens in these mutant mice have revealed that this gene is involved in avoidance learning, spontaneous locomotor behavior (Kinney et al. 2001a), and memory retention (Kinney et al. 2001b). As an example of the quantitative trait loci (QTL) approach, Peripato et al. (2002) showed that genes involved in the mouse behaviors that determine successful offspring survival, such as maternal aggression and pup retrieval, were localized to two chromosomal regions consisting of many small interacting QTL. They further identified many possible candidate genes to be screened for whether they affect these behaviors. These examples highlight the trend to use phenotypically abnormal animals with either genetic mutations or abnormal behaviors to illustrate the genetic basis and evolvability of behavior.

One behavior of great evolutionary significance is locomotion (Irschick and Garland 2001). Locomotion is involved in foraging, escaping from predators, finding mates, and caring for young. Indeed, Dickinson et al. (2000, p. 100) have claimed that “locomotion. . . is the behavior that most dictates the morphology and physiology of animals.” Wheel

running has often been used to study the locomotor behavior of rodents in the laboratory. Running propensity varies greatly among species of both laboratory-bred and wild-caught rodents (reviewed in Sherwin 1998). A genetic basis for the variability in wheel running has been demonstrated both in terms of its narrow-sense heritability ($h^2 = 0.20$; Oliverio et al. 1972; Swallow et al. 1998) and its significant dominance genetic variance for higher wheel running (Bruell 1964; Dohm et al. 1994). However the specific genes that determine levels of wheel running are not known. Genes that affect motivation for wheel running, exercise capacity, or perception of the aversive effects of exercise (e.g., pain) might determine levels of voluntary wheel running within and among species.

We conducted a selective breeding experiment to increase voluntary wheel-running behavior in four replicate lines of house mice (S lines), while maintaining four additional lines through random breeding to serve as controls (C lines; Swallow et al. 1998). The selection was quite successful, producing a 2.6-fold increase in running in S as compared to C mice, and a twofold increase compared to wild house mice (Dohm et al. 1994; Garland 2003). Levels of running have diverged such that control animals run on average 5–6 km/day, whereas selection males and females run on average 14–18 km/day. The change in wheel running with selective breeding encompasses the full range of running levels seen among species of rodents not subjected to behavioral selection (i.e., 5–18 km/day; Garland 2003). To date, few exercise-related genetic adaptations have been identified in S mice (Houle-Leroy et al. 2000; Dumke et al. 2001; Garland et al. 2002; Girard and Garland 2002; Houle-Leroy et al. 2003) suggesting that the alteration in behavior has primarily resulted from changes at the level of the central nervous system (CNS; Rhodes et al. 2001, 2003a).

The hippocampus is one of many regions of the CNS that may have evolved in S mice to facilitate the increased voluntary running. Neuronal activity in the hippocampus is strongly correlated with levels of voluntary wheel running among individuals within the S and C lines (Rhodes et al. 2003b). In rats, brain activity in the hippocampus is necessary to induce spontaneous wheel-running behavior, and the frequency of the brain activity is closely correlated with wheel-running speed (Slawinska and Kasicki 1998). In addition, wheel running causes new neurons to form in the hippocampus, suggesting that the hippocampus plays a functional role in wheel running (Rhodes et al. 2003a). Finally, S mice display impaired performance in the Morris water maze test, a task that depends on normal function of the hippocampus, suggesting that the physiology of the hippocampus has been altered in S mice (Rhodes et al. 2003a).

The aim of this study was to identify differences in gene expression of the hippocampus in S versus C mice that may predispose or allow the S mice to perform high levels of voluntary wheel running. We report here the gene expression profiles of the hippocampus in S and C mice, using high-density oligonucleotide microarrays, and discuss the relevance of the genes whose expression levels have changed as a result of selective breeding.

MATERIALS AND METHODS

Animals

Mice in this report were females from the 27th generation of a laboratory selective-breeding experiment designed to study the evolutionary correlates of exercise behavior (Swallow et al. 1998). The selection colony was begun from outbred, genetically heterogeneous Hsd:ICR mice (*Mus domesticus*) purchased from Harlan Sprague Dawley (Indianapolis, IN, Building 202, Barrier A). Four replicate lines (10 families each) were selectively bred each generation based on total number of revolutions run on days 5 and 6 of a six-day running-wheel exposure at approximately 8–10 weeks of age. Four replicate lines were randomly bred with respect to wheel running each generation. At generation 27 of selective breeding, two nonsibling females from each of the eight lines ($n = 16$ total) were weaned and placed individually in regular rodent cages without access to running wheels. All females used were chosen in the estrus phase of cycling as determined by a vaginal lavage (Drazen et al. 1999) to reduce experimental noise due to hormonal cycling. Rooms were controlled for temperature (about 22°C) and photoperiod (12:12 L:D; lights on at 0400 h, Central Standard Time).

Tissue Collection

At two months of age, mice were killed by cervical dislocation and then decapitated. The whole brain was removed and the hippocampus dissected out on top of an ice-filled glass petri dish under a dissecting scope. Tissues were weighed and placed at -80°C . All dissections were performed between 1300 and 1500 h over three consecutive days. An equal number of S and C mice were dissected each day. The entire hippocampal transcriptome from each female was used in this study, and was subjected to total RNA extraction using the guanidinium isothiocyanate method (TRIZOL Reagent, Life Technologies, Grand Island, NY).

Several steps were taken to reduce the confounding influence of physical activity itself on gene expression and to reduce among-animal variability: (1) brains were harvested during late stages of the light cycle, a time of low locomotor activity for mice (Naylor et al. 2000; Girard et al. 2001; Girard and Garland 2002); (2) only virgin females in the estrous phase of cycling were used; and (3) the animals had no exposure to running wheels from birth through death. Furthermore, to guard against measuring the possible effect of physiological stress due to running prevention in the S mice, we sacrificed at precisely the age at which mice are normally placed on running wheels in the selection experiment.

High-Density Oligonucleotide Array Hybridization

Each mouse hippocampal transcriptome was assayed individually, one oligonucleotide array per mouse hippocampus. All messenger RNA present in 10 μg of total RNA per individual were converted to double-stranded cDNA (Superscript Choice System, Life Technologies) and used as templates to synthesize biotin-labeled cRNA (T7 Megascript kit, Ambion, Austin, TX). Biotin-labeled cRNA was purified using RNeasy affinity columns (Qiagen, Valencia, CA). We

hybridized cRNA to high-density mouse oligonucleotide arrays (MU74Av2, Affymetrix, Santa Clara, CA) as described (Bronikowski et al. 2003). After hybridization, the gene arrays were washed and stained in a fluidic station (P/N no. 800101, Affymetrix) and scanned at a resolution of 6 μm with a Hewlett-Packard GeneArray Scanner (P/N no. 900154, Affymetrix).

Preliminary Data Analysis by Affymetrix Algorithms

The Affymetrix MG-U74Av2 array contains 12,422 probe sets representing 11,904 known or putative genes. Approximately 16 probe pairs of oligonucleotide probes in a probe set (16 perfect match and 16 mismatch probes) are used to measure the transcript level of a gene. Each probe pair consists of a perfect match (PM) probe and a mismatch probe (MM), which allows direct subtraction of cross-hybridization signals after background subtraction. GeneChip Analysis Suite 4.1 (Affymetrix Corp., Santa Clara, CA) was used to quantify the image data. Affymetrix software determines the presence of mRNA in samples and computes the signals of probe sets. The software calculates differences and ratios between perfect match and mismatch signals, which are representative of the hybridization levels of their targets in each probe set. The average of the differences between perfect match and mismatch signals (after removing the outliers beyond three standard deviations) is used to estimate relative mRNA levels of the transcripts. Global scaling normalizes signals in each image, in which the average signals of all probe sets in an image are scaled to the target average intensity by multiplying a scaling factor. We used the resulting variable, an individual's probe set signal intensity (signal intensity) for each gene, as the dependent variable in all statistical analyses. In our results tables, we also include the direction and percent change in average signal intensity from control to selection individuals. Positive percent change indicates that a gene was significantly induced in the selection mice, whereas negative percent change indicates that a gene was significantly repressed in the selection mice.

Statistical Analysis

We used a three-step inferential procedure to determine genes that were significantly affected by selection. First, we applied a conservative definition of gene expression to the approximately 12,000 genes that were analyzed. To warrant inclusion in subsequent analyses, a gene had to be expressed in all eight individuals in at least one of the two selection groups. A total of 3,439 genes met this criterion. Second, these genes, less the three genes chosen for a priori tests (see below), were subjected to a false discovery rate query (SAM algorithm; Tusher et al. 2001). This procedure allowed us to identify the false discovery rate of the list of genes we present: a 10% false discovery rate was achieved by using a nominal P -value cutoff of 0.018 in a simple t -test comparing the mean signal intensity of S versus C. Of 3,436 genes, 53 met this criterion. An additional 85 genes would have been included if we used the traditional cutoff P -value of <0.05 . These 85 genes are not discussed herein, but are listed in the supplementary table (available online at <http://dx.doi.org/10.1554/04-102.1.s1>) with their t -test levels of significance.

Third, because the lines were separately propagated for 27 generations, individuals in a given generation do not represent independent data points (i.e., individuals within a line are genetically more similar to one another than to individuals from other lines). Therefore, the lines must be nested within the populations to which they belong. To satisfy this requirement, the signal intensities of the 53 genes were analyzed using a mixed model ANOVA (SAS ver. 8.2, Proc GLM, SAS Institute, Cary, NC) with the fixed effect of line type (S versus C) and the random effect of line nested within line type (four S lines, four C lines). The F -test for an effect of selection divides the type III mean square for line type ($df = 1$) by the mean square for line nested within line type ($df = 6$; e.g., Bronikowski et al. 2001). The resulting probabilities associated with these F -tests are reported in Table 2.

We predicted that expression of dopamine receptors would differ in S versus C mice because S mice respond differently to several dopamine drugs including Ritalin, cocaine, GBR 12909, and SCH 23390. Therefore, the signal intensities of the dopamine receptor genes on the chip (DR2, 3, and 4) were subjected to a priori analyses with a two-tailed test of significance at the $P < 0.05$ level. These three genes were analyzed with the mixed model ANOVA described above.

RESULTS

Wheel Running

The 16 female mice used in this study (eight S, eight C; two from each of the lines) were randomly sampled from generation 27 of the selection experiment and were never exposed to running wheels. However, the other female members of this generation ($n = 271$), not used in this study, were measured for wheel running as part of the regular selection protocol (as described in Swallow et al. 1998). Figure 1 shows generational mean wheel running across all 27 generations of the experiment for all eight lines. As in Garland et al. (2002), a nested ANCOVA was used to compare mean wheel revolutions on days 5 and 6 of the six-day test (SAS Proc MIXED: line nested within line type, family nested within line, covariates of age and wheel freeness). As expected, line type had a highly significant effect ($F = 95.4$; $df = 1,6$; $P < 0.0001$), with adjusted means (\pm SE) of 13,473 (589.9) revolutions/day for S mice and 4,544 (697.7) for C mice. (Age had a significant negative effect [$F = 10.4$; $df = 1,200$; $P = 0.0014$] but wheel freeness had no significant effect [$F = 0.2$; $df = 1,200$; $P = 0.6532$].)

Body and Brain Masses

Summaries of body, brain, and hippocampus mass are found in Table 1. Nested ANOVAs indicated no significant differences between S and C mice for any of these traits, and nested ANCOVAs (body mass as covariate) confirmed these results (not shown).

Gene Expression in the Hippocampus

Of the 11,904 genes on the chip, 3,436 (about 29%) were expressed at a measurable level in all individuals from at least the S or C lines. Thus, there were 3,436 genes for which we conducted a test comparing expression levels between S

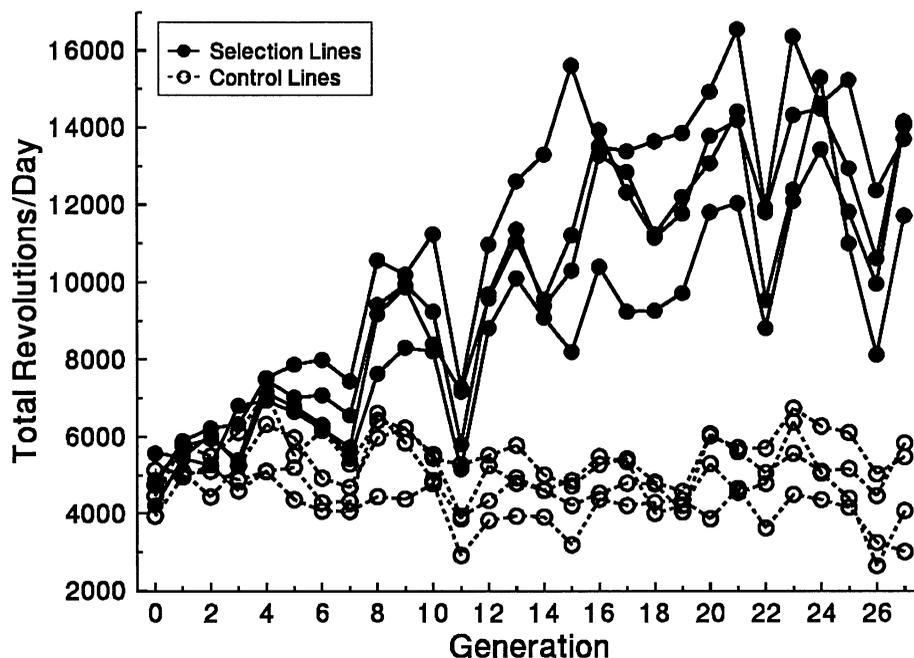


FIG. 1. Generational mean wheel running for females from eight lines of mice, four selectively bred for high voluntary wheel running and four bred randomly as controls. Values are mean revolutions on days 5 and 6 of a six-day exposure to running wheels (1.12 m circumference) as part of the routine selection protocol (see Swallow et al. 1998). For each generation, sample sizes are approximately 20 for each control line and 50 for each selection line. Mice for the present study were sampled from generation 27 and were not exposed to running wheels.

and C. Of these genes, 53 (about 2%) showed significant differences between S and C with a false discovery rate of 10%. Of these 53 genes, 30 displayed increased expression whereas 23 displayed decreased expression in S relative to C. Of the 30 genes that displayed increased expression in S versus C, the percent change relative to levels observed in C lines varied from 6–190%. These genes and their adjusted *P*-values from mixed model ANOVA are reported in Table 2. The largest categories of genes showing increased expression in S versus C were involved in transcription and protein biosynthesis, and neuronal signaling (Table 2). The 23 genes that displayed significantly decreased expression in S versus C mice were distributed across many categories (transcription, protein biosynthesis, immune response, cell signaling; see Table 2). These changes were also of relatively small magnitude (range = –10% to –68% of levels observed in C lines).

Because we had a priori evidence that dopamine gene expression might differ in S versus C mice (Rhodes and Garland 2003), we subjected the dopamine receptor expression levels to two-tailed a priori tests of significance at the $P < 0.05$ level. Of the three dopamine receptors that were present on the Affymetrix U74Av2 array (DR2, 3, and 4), DR3 was not

expressed in the hippocampus. Expression of DR4 and DR2 were 24% and 19% higher in S than C, respectively, and the difference for DR2 was marginally not significant (for DR4, $F_{1,6} = 5.88$, $P = 0.05$; for DR2 $F_{1,6} = 3.78$, $P = 0.10$).

DISCUSSION

This is the first study to demonstrate the molecular evolution of a brain region in response to known selection on a trait of major evolutionary significance, locomotor activity. Voluntary wheel running was the trait subjected to artificial selection in the laboratory and the hippocampus was chosen as the brain region for analysis because it is known to exhibit large physiological responses to wheel running. For example, running-induced neuronal activity in the hippocampus is associated with increased concentration of the transcription factor, Fos, the production of neuroprotective proteins, such as BDNF, and the formation of new neurons (Rhodes et al. 2003a). Based on these observations, we hypothesized that mice from lines that had been selectively bred for high wheel running would display adaptations in the hippocampus to predispose or enable these physiological responses to occur at a heightened level.

Increased Expression of Genes Involved in Transcription and Translation in the S Hippocampus

Several genes involved in transcription regulation and translation initiation displayed increased expression in the S mice. One of these transcription regulatory genes, mafG, functions to activate transcription of genes involved in neurogenesis (Shavit et al. 1998). Recently, we demonstrated

TABLE 1. Body mass, brain mass, and hippocampus mass for mice from selection and control lines.

| Avg (SD): | Body mass (g) | Brain mass (g) | Hippocampus mass (mg) |
|-----------|---------------|----------------|-----------------------|
| Selection | 23.4 (1.71) | 0.48 (0.02) | 47.2 (7.61) |
| Control | 25.1 (2.42) | 0.49 (0.02) | 46.5 (8.79) |

that neurogenesis occurs at a similar rate in S and C mice when they are housed without wheels (Rhodes et al. 2003a). Therefore, it is unlikely that the increased expression of mafG stimulated increased neurogenesis in the S mice of this study because they also were housed without wheels. Perhaps mafG interacts with other substrates released during exercise to increase neurogenesis. An intriguing possibility is that the increased expression of mafG in S versus C mice is an adaptation to prepare the S mice for the high amount of neurogenesis that occurs when the mice are allowed to run. The possible roles of other transcription factors that were upregulated in S mice, such as Nor Ixr-beta, cope, baf53a, and tar DNA binding protein, are not known at this time but may represent other adaptations to prepare the hippocampus of S mice for the effects of heightened activity during wheel running. Many genes involved in translation initiation and protein modification displayed increased expression in the S mice. The combination of increased expression of transcription factors coupled with increased translation suggests a higher degree of protein synthesis in the hippocampus of S mice without concomitant protein breakdown.

Decreased Expression of Genes Involved in Neuron Signaling and Innate Immunity in S Mice

The number of genes that decreased in expression as a result of selective breeding were both fewer in number and of smaller average percent change compared with those genes whose expression increased (see Table 2). Several of these genes are involved in neuron signaling. Tong et al. (2001) reported that expression of neuron signaling genes in rat hippocampus is increased as a result of exercise. Thus, the gene expression profile of the hippocampus that may facilitate or predispose high levels of exercise is not the same as that which is induced by exercise itself.

That S mice hippocampuses have decreased levels of certain inflammation immune-response genes relative to C mice suggests that either selective breeding entails a cost with respect to the ability to fight inflammation or that S mice may be more resilient to inflammation without necessitating the need for the innate immune response. With respect to the first possibility, both male (Bronikowski et al. 2003) and female (P. A. Carter, unpubl. data) S mice with wheel access live longer than S and C mice without wheels. Furthermore, middle-aged S mice with wheel access exhibit levels of antioxidant enzyme expression similar to those in matched-age S and C mice without running wheels, whereas exercising C mice have induced antioxidant enzyme expression (Bronikowski et al. 2002). This indicates that the antioxidant defense system is not induced in exercising S mice. Ultimately, this demonstrates that lower inflammation/immune response in S versus C mice in both brain (this study) and other tissue is not costly, at least in terms of life span. Additional studies on the basal and challenge activation of B and T cells and antioxidants in these mice will specifically address whether S mice exhibit alterations in their resilience to infectious disease.

DR2 and DR4 Dopamine Receptors in Increased Voluntary Wheel Running

Previous pharmacological studies have implicated the dopamine system in the high levels of wheel running in the S mice (Rhodes et al. 2001; Rhodes and Garland 2003). Dopamine plays an important role in motivation and we have hypothesized that altered dopamine function may underlie the increased motivation for wheel running in the S mice. However, the hippocampus is not usually considered to be the site at which dopamine acts to motivate behavior. Rather, regions such as the prefrontal cortex, nucleus accumbens, and caudate-putamen complex are traditionally considered to function in this capacity. These regions receive dense dopaminergic innervation compared with the hippocampus. Nonetheless, the finding that DR2 and DR4 may have increased expression in the hippocampus of S relative to C mice is intriguing. Human linkage and association studies (e.g., Grady et al. 2003, reviewed in DiMaio et al. 2003) have implicated a specific allelic variant of the DR4 receptor with attention deficit hyperactivity disorder (ADHD). We have argued previously that the S lines may represent a useful model of ADHD and in this capacity these results provide supportive evidence for an association between DR4 expression and genetic hyperactivity. To the best of our knowledge, the DR4 receptor does not display allelic variation in the mouse. Therefore, altered regulation in its transcription underlies the increased expression of DR4 in our mouse model.

The DR2 receptor has been implicated in drug addiction (Volkow et al. 2002), and recently we have argued that the S mice may represent a group of animals that are “addicted” to or “dependent” on the hypothetical natural reward that is produced by wheel running to the point where they compulsively seek the reward (Rhodes et al. 2003a). However, as stated before, the hippocampus is not traditionally considered to be the site at which the DR2 receptor acts to elicit drug reward or motivation. Moreover, it was the D1-like receptors (DR1 and DR5) not the D2-like receptors (DR2, DR3, and DR4) that were implicated by pharmacology in causing the high levels of running in the S mice. D1-like receptors were not present on the chip, and are not known to be expressed in the hippocampus. The fact that the S and C mice responded similarly to agents that block the D2 family of dopamine receptors suggests that if the gene expression of the D2-like receptors are altered in S mice, then the difference may not be functionally significant. Recently, we demonstrated that neurons in the prefrontal cortex, nucleus accumbens, and caudate-putamen (regions rich in dopamine receptors) become very active when mice are prevented from running, suggesting that these regions, under the control of dopamine, may indeed play an important role in motivation for wheel running in our model (Rhodes et al. 2003b). Therefore, it will be important to study the gene expression profile of these brain regions in the future.

We have presented a large-scale assay of transcriptional changes in the hippocampus of mice that have been selectively bred for high voluntary wheel running for 27 generations. Direct selection is a powerful force that can lead to both adaptation and speciation, and this study provides a rare

TABLE 2. Significantly affected genes (10% false discovery rate) in the selectively bred genetic background relative to control. GenBank accession numbers are listed under ORF; percent change is the change from control to selection signal intensity; *P* is the probability value associated with the *F*-test from mixed model analysis-of-variance (see Materials and Methods).

| ORF | Percent change | <i>P</i> | Gene | Function |
|---|----------------|----------|--|--|
| Cell division (3) | | | | |
| U50406 | 190 | 0.0009 | FADD, Fas associated via death domain | apoptotic adaptor molecule, Apoptosis regulation |
| AA032310 | 50 | 0.01 | SMC4 | structural maintenance of chromosomes in mitosis |
| M92420 | 36 | 0.02 | Transforming growth factor alpha | cell cycle regulation |
| CNS—General (2) | | | | |
| L20942 | 44 | 0.04 | Myelin-oligodendrocyte glycoprotein | myelin sheath component |
| U48398 | -58 | 0.0048 | Aquaporin 4 | water transport |
| CNS—Neuron signaling (7) | | | | |
| AI850277 | 57 | 0.05 | Neuromedin U25 | neuropeptide signaling, Unknown in brain |
| AF049124 | 53 | 0.01 | Neuronal pentraxin 2 | neuronal cell adhesion |
| AI841076 | 6 | 0.01 | Fez1 (Zygin1) | axonal outgrowth guidance machinery |
| AF029347 | -36 | 0.005 | Chloride channel protein 3 | ion transport |
| L25274 | -27 | 0.003 | CD166 antigen | neurite extension cell adhesion molecule |
| M62374 | -27 | 0.04 | Gamma aminobutyric acid receptor | neuronal inhibition |
| X95818 | -13 | 0.01 | Synaptophysin | vesicle targeting in synapse signaling |
| Immune and inflammatory response (6) | | | | |
| AA408180 | 36 | 0.03 | Glycoprotein CEA4b | immunoglobulin superfamily |
| AI854235 | 31 | 0.01 | RS21-C6 | T-cell receptor-CD3 complex expression |
| M35247 | -52 | 0.02 | Histocompatibility 2, T region locus 17 | defense response |
| M80206 | -42 | 0.02 | Poliovirus receptor related protein 2 | immunoglobulin V type |
| L25274 | -27 | 0.003 | Activated leukocyte cell adhesion | signal transduction, Immunoglobulin domain |
| AI836509 | -10 | 0.003 | Hspc alpha | induced by stress, molecular chaperone |
| Metabolism (5) | | | | |
| AW122052 | 68 | 0.05 | Sialic acid synthase | catalyzes PEP and ManNAc in energy metabolism |
| U16297 | 58 | 0.04 | Cytochrome b561 | mitochondrial electron transport |
| U56734 | -28 | 0.033 | Mrc2, Mannose receptor | electron transport |
| AA690483 | -21 | 0.05 | N-acetylglutamate synthase | amino acid metabolism |
| AW121960 | -18 | 0.03 | Inositol monophosphatase | carbohydrate metabolism |
| Protein biosynthesis (8) | | | | |
| AI429868 | 74 | 0.03 | Ligatin | translation initiation factor |
| X65922 | 53 | 0.03 | Ubiquitin-like Fau | ribosomal protein S30 |
| AA673574 | 38 | 0.03 | Galnt4 (glycosyl transferase) | part of protein processing Golgi apparatus |
| AI850546 | 43 | 0.03 | Signal sequence receptor | binds proteins to be processed in ER |
| AW060297 | 18 | 0.02 | Mitochondrial ribosomal protein L43 | translation |
| AA798971 | -36 | 0.02 | gene trap PAT12 | ER targeting protein |
| AI265655 | -29 | 0.02 | Cyclophilin-like 2 | protein folding |
| AW123979 | -23 | 0.002 | Eukaryotic Translation Initiation Factor 5 | translation initiation factor |
| Signal transduction (6) | | | | |
| M35662 | 53 | 0.05 | Somatotropin | growth hormone |
| AF022811 | 51 | 0.01 | Cornichon homologue | EGF signaling |
| AW050305 | 23 | 0.004 | Casein kinase 1 | protein kinase |
| J05185 | -68 | 0.02 | Prolyl 4 hydroxylase | thyroid hormone binding protein (p55) |
| X94404 | -19 | 0.04 | b3 for alpha3 subunit of L-type Ca2+ | calcium channel signaling |
| L40934 | -12 | 0.02 | Rab acceptor 1 | small GTPase signaling |

TABLE 2. (Continued)

| ORF | Percent change | P | Gene | Function |
|-------------------------------------|----------------|--------|---|---|
| Transcription regulation (9) | | | | |
| AB009693 | 92 | 0.02 | mafG | transcription activation in neurogenesis |
| AV160842 | 91 | 0.05 | Nuclear orphan receptor Ixr-beta | transcription factor |
| AW049031 | 45 | 0.01 | Core promoter element binding | transcription activation |
| AF041476 | 31 | 0.02 | BAF53a | chromatin-binding protein |
| AW210064 | 20 | 0.04 | TAR DNA binding protein | transcription regulation |
| AI837887 | 16 | 0.008 | SKD3 | transcription regulation, induced by stress |
| U53586 | -61 | 0.02 | Ecotropic viral integration site 5 | zinc finger transcription factor |
| AF042799 | -50 | 0.02 | Suppressor of white apricot (homologue) | RNA splicing factor |
| D10627 | -23 | 0.0009 | Zinc finger protein | transcription regulation |
| Unknown (7) | | | | |
| C80197 | 94 | 0.03 | EST | unknown |
| AV349827 | 61 | 0.006 | EST | unknown |
| AI648831 | 50 | 0.03 | EST | unknown |
| AI843426 | 30 | 0.02 | EST | unknown |
| AI840615 | 15 | 0.005 | EST | unknown |
| AW125168 | -41 | 0.009 | EST | unknown |
| AW123781 | -23 | 0.05 | EST | unknown |

“snapshot” of transcription changes that might occur during these processes. Overall, some genes increased expression ($n = 30$) and others decreased expression ($n = 23$) in association with selection for high wheel running, but these changes were relatively small in magnitude compared with changes induced by exercise itself (e.g., Carter et al. 2001; Tong et al. 2001). The magnitude of expression changes may have been larger in areas such as the prefrontal cortex that are known to be involved in motivation. Despite the small number of differences in gene expression, the finding of a high percentage of changes in transcription factor expression suggests a mechanism by which small changes in gene expression could support large changes at the level of phenotypic evolution. Although we currently do not know whether gene expression changes are consistent across other brain regions (e.g., cortex, striatum), it will be valuable to gain this information to understand fully how changes in gene expression across the CNS support behavioral evolution.

Our findings contribute to a growing literature that indicates the importance of small changes in the expression of a few genes for large changes in behavior. For example, mutant screens, and the analysis of the modulation of the expression of these single mutant alleles, have revealed altered behavioral phenotypes involved in courtship and learning behaviors in *Drosophila* (e.g., *fruitless* and *cacophony*, reviewed in Greenspan 1997), and demonstrate that the regulation of transcript production need be altered only slightly to disrupt normal behaviors. Robinson and Ben-Shahar (2002) have reviewed genes whose altered brain expression patterns, some of small magnitude, can explain changes in social behaviors such as monogamy in voles or foraging in honeybees. Recent exciting work in this area has been reported by Robinson and colleagues on the honeybee (Whitfield et al. 2003). They showed that the shift in behavior from brood caregiver to forager can be predicted at the individual level by assaying brain transcriptomes. Furthermore, they were able to factor out age and show that environmentally induced behavioral change caused the gene expression changes, many of which were of small magnitude. These studies considered either single-gene mutations and their expression effects on behavior, or expression assays of an evolved behavioral sequence. We have taken the complementary approach of applying selection to a behavior and asking what gene expression changes have evolved in concert. Together, these findings highlight the role for small changes in gene expression in evolutionary processes.

ACKNOWLEDGMENTS

We are grateful to I. Girard and the veterinarians of the Research Animal Resources Center at the University of Wisconsin for their technical assistance. This research was supported by grants from the National Science Foundation (IBN-0212567 to TG) and the National Institutes of Health (AG18922 to TAP, AG05784 to AMB) and by University of Wisconsin Graduate School and Department of Zoology start-up funds to SCG. The authors also wish to thank C. Barlow, T. Carter, and J. A. Del Rio of the Salk Institute for providing training in mouse brain microdissections.

LITERATURE CITED

- Anholt, R. R. H., C. L. Dilda, C. Sherman, J.-J. Fanara, N. H. Kulkarni, I. Ganguly, S. M. Rollmann, K. P. Kamdar, and T. F. C. Mackay. 2003. The genetic architecture of odor-guided behavior in *Drosophila*: epistasis and the transcriptome. *Nat. Genet.* 35:180–184.
- Bartke, A., H. Brown-Borg, J. Mattison, B. Kinney, S. Hauck, and C. Wright. 2001. Prolonged longevity of hypopituitary dwarf mice. *Exp. Gerontol.* 36:21–28.
- Bronikowski, A. M., P. A. Carter, J. G. Swallow, I. A. Girard, J. S. Rhodes, and T. Garland, Jr. 2001. Open-field behavior of house mice selectively bred for high voluntary wheel running. *Behav. Genet.* 31:309–316.
- Bronikowski, A. M., T. J. Morgan, T. Garland, Jr., and P. A. Carter. 2002. Anti-oxidant gene expression in active and sedentary house mice selected for high voluntary wheel-running behavior. *Genetics* 161:1763–1769.
- Bronikowski, A. M., P. A. Carter, T. J. Morgan, T. Garland, Jr., N. Ung, T. D. Pugh, R. Weindruch, and T. A. Prolla. 2003. Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart. *Physiol. Genomics* 12:129–138.
- Bruell, J. H. 1964. Heterotic inheritance of wheel running in mice. *J. Comp. Physiol. Psychol.* 58:159–163.
- Carter, T. A., J. A. Del Rio, J. A. Greenhall, M. L. Latronica, D. J. Lockhart, and C. Barlow. 2001. Chipping away at complex behavior: transcriptome/phenotype correlations in the mouse brain. *Physiol. Behav.* 73:849–857.
- Dickinson, M. H., C. T. Farley, R. J. Full, M. A. R. Koehl, R. Kram, and S. Lehman. 2000. How animals move: an integrative view. *Science* 288:100–106.
- DiMaio, S., N. Grizenko, and R. Jooper. 2003. Dopamine genes and attention-deficit hyperactivity disorder: a review. *J. Psychiatry Neurosci.* 28:27–38.
- Dohm, M. R., C. S. Richardson, and T. Garland, Jr. 1994. Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. *Am. J. Physiol.* 267:R1098–R1108.
- Drazen, D. L., S. L. Klein, A. L. Burnett, E. E. Wallach, J. K. Crone, P. L. Huang, and R. J. Nelson. 1999. Reproductive function in female mice lacking the gene for endothelial nitric oxide synthase. *Nitric Oxide* 3:366–367.
- Dumke, C. L., J. S. Rhodes, T. Garland, Jr., E. Maslowski, J. G. Swallow, A. C. Wetter, and G. D. Cartee. 2001. Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake. *J. Appl. Physiol.* 91:1289–1297.
- Garland, T., Jr. 2003. Selection experiments: an underutilized tool in biomechanics and organismal biology. Pp. 23–56 in V. Bels, J. Gasc, and A. Casinos, Eds. *Biomechanics and evolution*. BIOS Scientific Publishers, Oxford, U.K.
- Garland, T., Jr., M. Morgan, J. Swallow, J. Rhodes, I. Girard, J. Belter, and P. Carter. 2002. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. *Evolution* 56:1267–1275.
- Girard, I., and T. Garland, Jr. 2002. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *J. Appl. Physiol.* 92:1553–1561.
- Girard, I., M. W. McAleer, J. S. Rhodes, and T. Garland, Jr. 2001. Selection for high voluntary wheel-running increases speed and intermittency in house mice (*Mus domesticus*). *J. Exp. Biol.* 204:4311–4320.
- Grady, D. L., H. C. Chi, Y. C. Ding, M. Smith, E. Wang, S. Schuck, P. Flodman, M. A. Spence, J. M. Swanson, and R. K. Moyzis. 2003. High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. *Mol. Psychiatry* 8:536–545.
- Greenspan, R. J. 1997. A kinder gentler genetic analysis of behavior: dissection gives way to modulation. *Curr. Opin. Neurobiol.* 7:805–811.
- Houle-Leroy, P., T. Garland, Jr., J. G. Swallow, and H. Guderley. 2000. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. *J. Appl. Physiol.* 89:1608–1616.
- Houle-Leroy, P., H. Guderley, J. G. Swallow, and T. Garland, Jr. 2003. Artificial selection for high activity favors mighty mini-muscles in house mice. *Am. J. Physiol. Regulatory Integr. Comp. Physiol.* 284:R433–R443.
- Irschick, D. J., and T. Garland, Jr. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annu. Rev. Ecol. Syst.* 32:367–396.
- Kinney, B. A., C. J. Meliska, R. W. Steger, and A. Bartke. 2001a. Evidence that Ames dwarf mice age differently from their normal siblings in behavioral and learning and memory parameters. *Hormones Behav.* 39:277–284.
- Kinney, B. A., K. T. Coschigano, J. J. Kopchick, R. W. Steger, and A. Bartke. 2001b. Evidence that age-induced decline in memory retention is delayed in growth hormone resistant GH-R-KO (Laron) mice. *Physiol. Behav.* 72:653–660.
- Naylor, E., B. M. Bergmann, K. Trauski, P. C. Zee, J. S. Takahashi, M. H. Vitaterna, and F. W. Turek. 2000. The circadian clock mutation alters sleep homeostasis in the mouse. *J. Neurosci.* 20:8138–8143.
- Oliverio, A., C. Castellano, and P. Messeri. 1972. Genetic analysis of avoidance, maze, and wheel-running behaviors in the mouse. *J. Comp. Physiol. Psychol.* 79:459–473.
- Peripato, A. C., R. A. de Brito, T. T. Vaughn, L. S. Pletscher, S. R. Matioli, and J. M. Cheverud. 2002. Quantitative trait loci for maternal performance for offspring survival in mice. *Genetics* 162:1341–1353.
- Rampon, C., C. H. Jiang, H. Dong, Y.-P. Tang, D. J. Lockhart, P. G. Schultz, J. Z. Tsien, and Y. Hu. 2000. Effects of environmental enrichment on gene expression in the brain. *Proc. Natl. Acad. Sci. USA* 97:12880–12884.
- Rhodes, J. S., G. R. Hosack, I. Girard, A. E. Kelley, G. S. Mitchell, and T. Garland, Jr. 2001. Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology* 158:120–131.
- Rhodes, J. S., and T. Garland, Jr. 2003. Differential sensitivity to acute administration of Ritalin, apomorphine, SH23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology* 167:242–250.
- Rhodes, J. S., H. van Praag, S. Jeffrey, I. Girard, G. S. Mitchell, T. Garland, Jr., and F. H. Gage. 2003a. Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behav. Neurosci.* 117:1006–1016.
- Rhodes, J. S., T. Garland, Jr., and S. C. Gammie. 2003b. Patterns of brain activity associated with variation in voluntary wheel-running behavior. *Behav. Neurosci.* 117:1243–1256.
- Robinson, G. E., and Y. Ben-Shahar. 2002. Social behavior and comparative genomics: New genes or new gene regulation? *Genes Brain Behav.* 1:197–203.
- Shavit, J. A., H. Motohashi, K. Onodera, J. Akasaka, M. Yamamoto, and J. D. Engel. 1998. Impaired megakaryopoiesis and behavioral defects in mafG-null mutant mice. *Genes Dev.* 12:2164–2174.
- Sherwin, C. M. 1998. Voluntary wheel running: a review and novel interpretation. *Anim. Behav.* 56:11–27.
- Slawinska, U., and S. Kasicki. 1998. The frequency of rat's hippocampal theta rhythm is related to the speed of locomotion. *Brain Res.* 796:327–31.
- Swallow, J. G., P. A. Carter, and T. Garland, Jr. 1998. Artificial selection for increased wheel-running behavior in house mice. *Behav. Genet.* 28:227–237.
- Tong, L., H. Shen, V. M. Perreau, R. Balazs, and C. W. Cotman. 2001. Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiol. Dis.* 8:1046–1056.
- Tusher, V. G., R. Tibshirani, and G. Chu. 2001. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA* 98:5116–5121.
- Volkow, N. D., J. S. Fowler, and G. J. Wang. 2002. Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. *Behav. Pharmacol.* 13:355–366.
- Whitfield, C. W., A.-M. Cziko, and G. E. Robinson. 2003. Gene expression profiles in the brain predict behavior in individual honeybees. *Science* 302:296–299.

Supplementary Table. Genes altered in the selection genetic background relative to controls whose significance level in a t-test was $0.018 < P < 0.05$ ($n = 85$). For genes with $P \leq 0.018$, see text ($n = 53$). Genbank Accession numbers are listed under ORF, Percent change is the change from control to selection signal intensity, and P is the probability value associated with a t-test.

| ORF | Percent Change | P | Gene | Function |
|--|----------------|------|---|--|
| CNS - NEURON SIGNALING (6) | | | | |
| AF043120 | 40 | 0.02 | Beta prime coatmer | Golgi binding and vesicle trafficking |
| AB025218 | 36 | 0.03 | Sid394p | Collects cargo molecules into budding vesicles |
| AW124144 | 26 | 0.04 | Evectin2 | Neuronal intracellular signaling cascade |
| X14972 | 20 | 0.03 | Adaptor related complex 2 | Vesicle trafficking |
| AA968123 | -49 | 0.02 | Neuronal Navigator 1 | Neuron growth and regeneration |
| D45208 | -38 | 0.02 | Syntaxin | Synaptic vesicle docking in synapse signaling |
| CNS – GENERAL (7) | | | | |
| AW047630 | 11 | 0.04 | Cern4L, carbon catabolite repression | Circadian rhythms |
| AI836916 | 7 | 0.03 | Nuclear phosphoprotein Anp32A | Modulates neurogenesis |
| X98014 | -37 | 0.02 | Sialyltransferase (alpha 2,8) | Ganglioside synthesis |
| U02982 | -28 | 0.05 | Secretogranin III | Neuronal intracellular vesicle secretory granule component |
| AF006467 | -25 | 0.04 | Phosphatidylinositol | Regulates phototransduction |
| U29088 | -22 | 0.04 | ELAV-like protein 2 | RNA binding protein in CNS |
| AI835461 | -18 | 0.04 | Aldh7a1, oxidoreductase | Metabolic pathway, involved in hearing |
| TRANSCRIPTION REGULATION (8) | | | | |
| AB023419 | 41 | 0.02 | Sox7 | Transcription suppression |
| U62906 | 27 | 0.04 | Zinc finger 94 | Transcription regulation |
| AF091234 | 26 | 0.03 | SMAR1 | btg3 associated transcription factor |
| M25773 | -63 | 0.03 | SWI/SNF, matrix associated | Transcription regulation |
| D70848 | -50 | 0.03 | Zinc finger protein cerebellum 2 | Transcription regulation |
| AA833293 | -36 | 0.04 | Forkhead box p1 | Negative regulation of transcription |
| U70736 | -16 | 0.04 | JUN activation binding protein 1 | Phosphorylation of transcription factors |
| AI852704 | -12 | 0.04 | Unnamed DNA binding protein | Negative Regulation of transcription from PolII |
| IMMUNE (4) | | | | |
| X57413 | 42 | 0.02 | TGF beta 2 | Suppresses interleukin-2 dependent T cell growth |
| AW120786 | -56 | 0.04 | Small inducible cytokine B14 | Regulates leukocyte infiltration into inflamed tissue |
| AV360742 | -39 | 0.04 | P24 | vesicle membrane protein similar to cyclophilin |
| AC002397 | -34 | 0.04 | T cell antigen T4 | MHC II/ T cell receptor interaction |
| CELL GROWTH/ TROPHIC (10) | | | | |
| AI153421 | 86 | 0.04 | Erythroid differentiation factor | Induces hemoglobin synthesis |
| J03520 | 69 | 0.04 | Plasminogen activator | Controls plasmin-mediated proteolysis |
| U57820 | 43 | 0.03 | MyoD family inhibitor | Development, muscle differentiation |
| X87128 | 27 | 0.04 | p75 TNF receptor | Cell growth and maintenance |
| Z19521 | 19 | 0.02 | Low-density lipoprotein receptor | Endocytosis, Binds LDL and transports into cells |
| AA790008 | 17 | 0.02 | Reduced expression 3 | Involved in cell growth arrest |
| D42048 | 13 | 0.04 | Squalene monooxygenase | Sterol biosynthesis |
| AI845814 | -59 | 0.03 | Zinc transporter Slc39a3 membrane protein | Metal ion transport into cell |
| L12367 | -34 | 0.04 | Adenyl cyclase associated protein | Cellular regulation |
| Y17345 | -25 | 0.04 | Protein tyrosine phosphatase IF1 | Negative regulation of cell proliferation |
| PROTEIN BIOSYNTHESIS/BREAKDOWN (10) | | | | |
| AI853036 | 42 | 0.03 | Calnexin | Retains incorrectly folded proteins in the ER |
| AW049793 | 38 | 0.03 | EIF4 gamma 2 | Translation initiation factor |
| AF038008 | 32 | 0.03 | Protein tyrosine sulfotransferase 1 | O sulfination of tyrosine residues, protein integrity |
| AV345874 | 31 | 0.04 | Mapre1 | Golgi Apparatus microtubule associated |
| M20632 | 17 | 0.04 | 40S ribosomal protein S2 | Translation |
| AI835460 | 15 | 0.02 | Interleukin enhancer binding factor 3 | Inhibits translation by binding mRNA |
| L25125 | -70 | 0.04 | ATP dependent RNA helicase DDX19 | Translation initiation |
| U38981 | -40 | 0.03 | Uterine protein | Intraflagellar transport protein involved in protein trafficking |
| AI852665 | -31 | 0.04 | Mki67ip, RNA binding protein | Post-transcription modification |
| U06944 | -31 | 0.02 | Praja1 | Protein trafficking |

SIGNAL TRANSDUCTION (8)

| | | | | |
|----------|-----|------|--|--|
| U08373 | 44 | 0.04 | Calmegin | Calcium signaling |
| AB025218 | 36 | 0.03 | Sid394p | Collects cargo molecules into budding vesicles |
| AF095642 | 35 | 0.04 | FSH receptor | Activates adenylate cyclase |
| AF010133 | 20 | 0.03 | SMAD6 | TGF beta signaling |
| U46155 | 18 | 0.03 | Patched protein 2 | Receptor for hedgehog, axon guidance |
| A1848598 | 17 | 0.03 | RAS related protein 1a | GTPase mediated signal transduction |
| AW050034 | -43 | 0.03 | Thyroid hormone receptor interactor 12 | Thyroid hormone signaling |
| U29055 | -21 | 0.03 | Guanine nucleotide-binding protein | General modulator in transmembrane signaling |

STRESS (4)

| | | | | |
|----------|-----|------|---------------------------------|--|
| AF004934 | 49 | 0.03 | Ste20 kinase | Serine threonine kinase induced by oxidative stress |
| AB027566 | 32 | 0.04 | Thioredoxin reductase 2 | Induced by reactive oxygen species (redox signaling) |
| A1098675 | 32 | 0.03 | Thioredoxin interacting protein | Induced by reactive oxygen species (redox signaling) |
| AW050240 | -28 | 0.02 | Casein Kinase2 | Protein Kinase CK2 complex, DNA damage induced |

OTHER (8)

| | | | | |
|----------|-----|-------|---|---|
| AJ006469 | 66 | 0.03 | Cartilage-associated protein | Cell integrity |
| L26320 | 39 | 0.03 | FLAP endonuclease 1 | DNA synthesis: Cleaves 5' FLAP structures |
| AJ228865 | 29 | 0.03 | CYLN2 | Mediates cell membrane organelle interactions with microtubules |
| A1551087 | 23 | 0.04 | Tropomyosin | Muscle contraction |
| AW125698 | 23 | 0.04 | Myosin Heavy Chain | Muscle contraction |
| AF026072 | 16 | 0.04 | Hydroxysteroid sulfotransferase | Brain specific steroid metabolism |
| A1850118 | -29 | 0.048 | Arpc5, Actin related protein 2/3complex, subunit5 | Actin filament organization |
| AA986258 | -15 | 0.04 | Antiquitin | Oxidoreductase that modulates turgor pressure |

UNKNOWN (20)

| | | | | |
|----------|-----|------|--|--|
| A1854265 | 60 | 0.02 | | |
| AV339602 | 46 | 0.03 | | |
| A1847423 | 43 | 0.04 | | |
| AA759910 | 38 | 0.04 | | |
| A1181906 | 38 | 0.02 | Bcas2, breast carcinoma amplified sequence2 | |
| A1852429 | 26 | 0.04 | | |
| AW047671 | 25 | 0.03 | | |
| AW060956 | 25 | 0.03 | | |
| AW045632 | 24 | 0.04 | | |
| A1852047 | 24 | 0.03 | | |
| AW121102 | 15 | 0.02 | Membrane protein | |
| AW125329 | -66 | 0.04 | | |
| C79015 | -50 | 0.04 | | |
| AA189811 | -35 | 0.04 | | |
| A1848699 | -29 | 0.04 | Membrane protein | |
| AW125086 | -28 | 0.04 | | |
| AW125418 | -28 | 0.02 | Nicolin1 | |
| A1851565 | -25 | 0.02 | | |
| A1643393 | -20 | 0.04 | | |
| A1648965 | -17 | 0.03 | Xenotropic and polytropic retrovirus receptor1 | |