Gene expression comparisons between captive and wild shrew brains reveal captivity effects

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Supplementary Material available at
URL: https://figshare.com/articles/figure/BiologyLetters_CaptivityRNA_Revision/27237300
DOI: https://doi.org/10.6084/m9.figshare.27237300.v1

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ABSTRACT

Compared to their free-ranging counterparts, wild animals in captivity experience different conditions with lasting physiological and behavioral effects. Although shifts in gene expression are expected to occur upstream of these phenotypes, we found no previous gene expression comparisons of captive vs. free-ranging mammals. We assessed gene expression profiles of three brain regions (cortex, olfactory bulb, and hippocampus) of wild shrews (*Sorex araneus*) compared to shrews kept in captivity for two months and undertook sample drop-out to examine robustness given limited sample sizes. Consistent with captivity effects, we found hundreds of differentially expressed genes in all three brain regions, 104 overlapping across all three, that enriched pathways associated with neurodegenerative disease, oxidative phosphorylation, and genes encoding ribosomal proteins. In the shrew, transcriptomic changes detected under captivity resemble responses in several human pathologies, including major depressive disorder and neurodegeneration. While interpretations of individual genes are tempered by small sample sizes, we propose captivity influences brain gene expression and function and can confound analyses of natural processes in wild individuals under captive conditions.

Keywords: Captivity, brain, shrew, transcriptomics.

INTRODUCTION

Wild animals placed in captivity are subject to altered environmental conditions in comparison to their free-ranging counterparts. Shelter, movement space, diet, climate, lighting, social factors, and human presence vary drastically between the wild and captivity [1–4]. Organisms respond to these changes by altering behavior, physiology, biochemistry and gene expression, but these responses occur at different time scales [5]. While long-term habituation to captivity can activate multiple stress responses, even short-term habituation to captive conditions affects brain morphology and function [1,3,6–8]. Symptoms of chronic stress appear because of frequent exposure to perceived threats [2] that with reduced environmental stimulation can lead to depressive-like states in captive animals [9]. Thus, despite the perception of a comfortable, predictable, and controlled environment, captive conditions can generate detrimental responses in captive individuals.

Short-term and long-term environmental changes also lead to lasting changes in gene expression, with a central role in cellular adaptation through extensive transcriptional and post-transcriptional regulation [10–13]. Although studies in mammals have addressed the effects of captivity in many phenotypes downstream of transcription, including morphology [14,15], longevity [16] and stress [17,18], to our knowledge, there are no experiments comparing brain gene expression in non-domesticated wild and captive mammals. This is of particular interest to modern research, as many experiments are performed on mammals in laboratory settings and may yield different results from those obtained in the wild [6,19], potentially confounding the effects of captivity with phenotypes of interest.

To explore potential changes in the brain from captivity, we assessed the brain gene expression profiles of wild common shrews (*Sorex araneus*) compared to same-age shrews held captive for two months (>10% of their lifespan). The common shrew is ideal for revealing potential captivity effects on gene expression, as many research resources are available: a complete genome assembly and a brain MRI-atlas with region-specific gene expression profiles [20]. *S. araneus* is also relevant to neuroscience for its seasonal change in brain size [21]. We focused on three brain regions that may shift functional importance in an environment that does not require exploring complex surroundings: 1) the cortex,

whose evolution in mammals has improved information processing and decision making, 2) the hippocampus, involved in spatial memory and learning, and 3) the olfactory bulb, which receives olfactory information from the environment. We outline differences in brain gene expression between wild and captive shrews detected using differential gene expression analysis of mRNA. Our results support a captivity effect in the shrew brain, and we highlight similar responses found in studies of metabolism, neurodegenerative diseases, stress, and depression.

MATERIALS AND METHODS

Shrew Collection

Shrews were caught in Liggeringen, Germany, as previously described [22] to reduce stress. Our study had small sample sizes, as only ten individuals from the population could be sampled in autumn and logistical barriers from the COVID-19 pandemic restricted sampling over multiple years. Captive juvenile shrews were caught in September of 2020 and housed individually for two months at the Max Planck Institute of Animal Behavior before samples were taken (Table 1). Animals were allowed to move freely between two connected cages, one containing a running wheel, bowls of food and water, a nesting pot, and the other consisting of several layers of soil and hay (detailed housing in [23]). Captive shrews were kept at ambient outdoor temperatures, ranging from -5°C to 22°C, and provided a daily diet of 5g of raw meat and 2g of mealworm. Only four autumn wild juvenile shrews (two female and two male) were caught in November, and were sampled the same day of capture. Although sex is used as a covariate in subsequent analyses, there were two female wild individuals, but no captive females. This confounded sex and captivity in experimental design. Season was used as a proxy for age, as *S. araneus* have an unusually short lifespan of ~13 months, with one reproductive season followed by senescence. Overlap between generations occurs in summer and early fall, when adults have worn teeth, disheveled fur, and developed gonads. Adults were excluded from the experiment.

Tissue Sampling, RNA Extraction, and Sequencing

Shrews were weighed, then anesthetized and euthanized under deep anesthesia by perfusion of the vascular system with PAXgene Tissue Fixative (perfusion described in Lázaro et al., 2018 [22]). The brain was extracted immediately, weighed, dissected into individual regions, and submerged in cold fixative. Three brain regions were compared between captive and wild individuals: cortex, olfactory bulb, and hippocampus. Brain regions were held in fixative, and within 24 hours were switched to PAXgene Tissue Stabilizer and frozen in liquid nitrogen. Skull toothrow length was measured to assess the size of individuals. RNA was extracted from each brain region using a modified Qiagen Micro RNAeasy protocol designed to retrieve RNA from small sensory tissues [24]. Quality control, library preparation, and sequencing were conducted by Genewiz. RNA integrity numbers (RIN) were measured with RNA ScreenTape and samples below a RIN of 6 were removed from the experiment. mRNAs were selected by polyA enrichment followed by paired-end sequencing with Illumina HiSeq.

Bioinformatics

Samples with low sequencing depths (<20 million reads) were removed from the experiment. Adapters were trimmed and low-quality reads removed with fastp 0.20.1 [25]. Reads were mapped by pseudo-alignment to the *S. araneus* reference transcriptome (GCF_000181275.1_SorAra2.0_rna) using Kallisto 0.46.1 [26]. Samples with mapping percentage under 45% were removed. Read counts were then

normalized for library size and content using the median of ratios from DESeq2 1.36.0 [27]. To visualize the overall effect, we ran a principal component analysis (PCA) of the 500 most varying genes for each brain region using variance stabilized read counts [28].

Differential gene expression between captive and wild individuals for each brain region was analyzed in R (version 4.2.0) with DESeq2 [27]. Sex and body size were used as additional covariates for the analysis (~sex+toothrow+condition). Maxillary tooth row length was used as a proxy for body size, as it does not change seasonally within individuals [29,30]. Statistical significance for each region comparison was determined by conducting a modified Wald test, using an alternative null of 20% change, and then adjusting p-values for multiple testing using Benjamini and Hochberg correction [31] ($p_{adj} < 0.05$). The proportion of true nulls was analyzed with limma [32]. Given a statistical significance threshold of 0.05, the expected proportion of true nulls approaches 0.95. Higher values indicate false negatives, and lower values indicate false positives. Genes identified as differentially expressed for each brain region were separated by direction (upregulated or downregulated). KEGG pathway enrichment analysis (Modified Fisher's Exact test) was carried out for both directional gene lists of each brain region using DAVID [33,34]. To examine result robustness, we conducted a drop-out analysis for each brain region (Supplemental Figures 1-3). We removed one individual per analysis, identified overlapping differentially expressed genes across all analyses, reran DAVID pathway enrichments, and identified overlapping pathways.

RESULTS

We identified divergence in the expression profiles of all three brain regions associated with a potential captivity effect. For each brain region, PCA produced two principal component axes that accounted for most of the variation from these genes (Figure 1). The first principal component separated wild from captive individuals, accounting for 42% of the variation in gene expression in the hippocampus and 47% in the olfactory bulb. Percent variation expressed was even greater in the cortex, where the first principal component accounted for 63% of the variation. As for PC2, each region highlighted one sample within each analysis with variation not found in other samples. We determined this was not a single outlier sample, as we found each case corresponded to different individuals for each brain region (Supplemental Figure 4), with no discernible pattern across regions.

Differential expression analysis confirmed these patterns (Figure 1). Of the 19,296 genes analyzed, 603 genes were differentially expressed (DEGs; $p_{adj}<0.05$) in at least one brain region (Figure 2). In the cortex, we found 434 DEGs, 162 upregulated and 272 downregulated in captive shrews. The olfactory bulb had 298 DEGs, with 185 upregulated and 112 downregulated in captive animals. In the hippocampus, we identified 180 DEGs, 48 upregulated and 132 downregulated in captive animals. The proportion of true nulls was high, 0.97 in the cortex, 1.00 in the hippocampus, and 0.99 in the olfactory bulb. This indicates lower statistical power to detect many true positives in the hippocampus and olfactory bulb because of small samples sizes and high variability [35,36]. We compared lists of DEGs between brain regions and found 104 genes that were differentially expressed in the same direction across all regions, with another 101 overlapping in two of three brain regions (Figure 2).

To partially overcome small sample sizes, we undertook pathway enrichment analyses, which revealed captive gene expression differences are linked to ribosomal and mitochondrial function, with associations to neurodegenerative processes (Figure 3). We identified 21 pathways significantly

159 enriched (p-value < 0.05, modified Fisher's Exact Test) for the cortex, 21 for the olfactory bulb and 21 for
160 the hippocampus. Out of these, 14 pathways were significantly enriched ($p<0.05$) across the three brain
161 regions (Figure 3), with the largest enrichment in the ribosome pathway (p_{cor}: 7.4×10^{-32}, n= 35genes; p_{olf}:
162 4.0×10^{-11}, n=13 genes; p_{hip}: 5.0×10^{-15}, n=17 genes). Within enriched pathways, we identified expression
163 changes that likely affect mitochondrial function in the brain. Nineteen genes encoding mitochondrial
164 complex I NADH:ubiquinone oxidoreductase subunit proteins (largest enzyme of the respiratory chain),
165 of which 8 overlap all three regions and were found across 12 of the 14 overlapping enriched pathways
166 (Figure 3). Six of the pathways containing complex I proteins were associated with neurodegenerative
167 diseases, including Parkinson's disease, amyotrophic lateral sclerosis, pathways of neurodegeneration-
168 multiple diseases, prion disease, Huntington's disease, and Alzheimer's disease. Out of the eight
169 enriched pathways remaining, six of them were associated with changes in metabolism, such as
170 oxidative phosphorylation, retrograde endocannabinoid signaling, non-alcoholic fatty liver disease,
171 diabetic cardiomyopathy, chemical carcinogenesis - ROS, and thermogenesis, highlighting the influence
172 of mitochondrial genes on both metabolically related and neurodegenerative mechanisms.

173 While principal component analyses show little impact from sex, drop-out analyses indicate robust gene
174 expression results in the cortex (Supplemental Figures 5 and 1-3). First, we examined the expression of
175 each individual mitochondrial gene of interest for each brain region (Supplemental Figures 6A-B).
176 Normalized counts did show some dependance on sex. But instead of driving differences in gene
177 expression, sex differences appear to dampen overall signal, and differences are greater between wild
178 and captive samples than between sexes. Pathway enrichment for drop-out analyses was robust for the
179 cortex, where 109 significant genes from the full experimental analysis remained differentially
180 expressed across all dropouts ($p_{adj}<0.05$). This subset of robust genes enriches most of the proposed
181 pathways, including oxidative phosphorylation, neurodegeneration, Parkinson's disease, Alzheimer's
182 disease, prion disease, Huntington's disease, and ALS ($p<0.05$). The olfactory bulb and hippocampus
183 were not robust to sample drop-out, as only 27 genes for the hippocampus and 15 genes for the
184 olfactory bulb remained across all drop-out analyses and did not enrich highlighted pathways.

185 DISCUSSION

186 Both genomic [37,38] and environmental variation [39–41] impact neural structures and function. Past
187 studies have focused on fixed domestication-associated neural changes in subsets of mammals [42–44],
188 and identified differences in gene expression and transcription factors involved in domestication-
189 associated changes in both brain and craniofacial phenotypes [37,38]. While brain gene expression
190 responses to captive environment change have been documented in diverse organisms [45–48], this is
191 the first study documenting potential captivity effects specifically on mammalian brain transcripts in the
192 absence of artificial selection. Although captivity is routine in many experiments, effects on expression
193 may parallel or counter responses to domestication or other forms of induced or natural environmental
194 change. Potentially large expression changes in the brain after a brief stint in captivity highlight the
195 importance of working with both wild and captive individuals to avoid confounding gene expression
196 changes from phenotypes of interest with those associated with captivity.

197 We found potential region-specific responses to captivity, but small sample sizes mean these results are
198 limited by low statistical power and should be interpreted with caution. Principal components separate
199 samples by captivity first (Figure 1) so that overall expression supports a captivity effect. Individual
200 DEGs, however, are not robust to sample drop-out for the underpowered hippocampus and olfactory

201 bulb (Supplemental Figure 2 and 3). For example, in the hippocampus we found apparent upregulation
202 of four myosin genes (*MYH6*, *MYL2*, *MYL3*, *MYBPC3*) (Figure 1). This may relate to changes in synaptic
203 plasticity, as myosins regulate actin cytoskeleton dynamics in dendrites essential for long-term
204 potentiation and memory formation in rodents [49–52]. But based on power analyses from vertebrate
205 brain expression [36], when sampling four individuals, there is ~70% power to accurately detect
206 differential expression in genes with more than 40 reads. While most observed DEGs are above this
207 threshold (Supplemental Figure 7), nearly half of the total genes sequenced had fewer than 40 reads,
208 reducing the power to detect differential expression (~5% to 45%), and therefore the reliability for each
209 individual differentially expressed gene.

210 Instead, we focus biological interpretations on pathway changes found across all three brain regions
211 (Figure 3), as gene sets reduce noise and are more stable compared to individual gene expression
212 changes [53]. We found downregulation of ribosomal protein genes in captive shrews. Ribosomes
213 compose the cell's protein synthesis machinery, and their own synthesis, ribosomal biogenesis, is a
214 highly conserved process [54,55]. In the captive shrew brain, downregulation of 12 ribosomal proteins
215 (RPs) suggests impaired regulation of ribosomal biogenesis and subsequent functions. By determining
216 translational capacity of proliferating cells [56], ribosomal biogenesis constrains animal growth and
217 development [57,58]. Dysregulation of ribosomal biogenesis is therefore implicated in numerous
218 phenotypes including neurite survival and synaptic function associated with human neurodegeneration
219 [54]. These effects seem to be mirrored in captive shrews, as we found downregulated gene enrichment
220 for pathways associated with neurodegeneration. Neurodegeneration has been linked to changes in
221 stress [59,60], but these transcriptomic profiles and potential shifts in processes such as reduced
222 synaptic efficiency might be occurring because of captivity and not disease.

223 Differential expression of ribosomal protein-coding genes in the brains of captive shrews could influence
224 behavior, as many such genes have been previously highlighted for their regulatory changes under
225 domestication and social defeat stress [44,61]. For example, comparisons between domestic rabbits and
226 wild counterparts identified 612 DEGs across four brain regions that also enriched ribosomal pathways
227 [44]. Additionally, changes in expression of ribosomal genes have also been associated with decreased
228 proliferation in the hippocampus of mice under chronic social defeat stress, which consists of repeated
229 negative social experiences (defeats) produced by daily agonistic interactions with an aggressive
230 partner, leading to states of anxiety and depression [61]. Captivity-associated differential expression of
231 ribosomal proteins in the shrew brain may thus indicate a depressed neural state, especially as past
232 studies have argued that animals in captivity often experience repeated stressors used to induce
233 depressive-like states [9].

234 Captivity conditions such as altered diet and physical activity may also influence shrew energy
235 metabolism in the brain. In captive shrews, we found downregulation of mitochondrial complex I
236 proteins, essential for energy production, as they comprise the first and largest complex of the electron
237 transport chain [62,63]. Impairments to complex I function are linked to pathophysiological states,
238 genetic diseases [62,64–66], and several neurodegenerative disorders, leading to decline of energy
239 production and increased production of reactive oxygen species [67–70]. Downregulation of 8 genes
240 encoding for mitochondrial complex I subunit proteins in three shrew brain regions suggests a
241 dysfunction of the oxidative phosphorylation (OXPHOS) system and dysregulation of energy metabolism
242 in captivity. These genes are also downregulated in the brains of patients with autism [71], and
243 neurodegenerative diseases such as Huntington's [72] and Alzheimer's [73]. Indeed, the effects of

244 certain conditions found in captivity, such as reduced stimulation, could also modify OXPHOS function in
245 association with neurodegeneration, as normal brains exposed to chronic sensory deprivation exhibit
246 downregulation of OXPHOS subunits as found in the brain of Alzheimer's patients [74]. However, studies
247 have found differences in mitochondrial function/gene expression between sexes [75–77], and sex-
248 dependent susceptibility to neurodegenerative disease [76]. Therefore, including only two female
249 samples is a limitation of our study to be improved in the future.

250 As with differential ribosomal protein expression, mitochondrial dysfunction and changes in expression
251 of complex I subunits have also been linked to depressed activity and stress-related behaviors. For
252 example, female mice bred for low activity showed upregulation of complex I subunit genes [78],
253 including three genes downregulated in the three brain regions tested in captive shrews (*NDUFA2,*
254 *NDUFA4,* and *NDUFS5*). These genes exhibit opposite expression patterns between low activity mice and
255 captive shrews. But other studies have found the direction of changes in mitochondrial gene expression
256 related to stress and anxiety may depend on stressor duration, genetic background, and strain of the
257 model organism studied [79]. Nevertheless, published and newly detected shifts still point to
258 mitochondrial pathway dysregulation as a major brain stress response [79]. Differential expression of
259 complex I subunit genes in captive shrews is consistent with a transcriptional phenotype associated with
260 depression and stress in model organisms. While the validation of specific mechanisms for such a
261 transcriptional phenotype (e.g., by western blot validation) is outside the scope of this paper, we have
262 identified key potential targets for future analyses.

263 Conclusion

264 Despite attempts to limit differential stressors and optimize holding conditions, these results indicate
265 transcriptomic changes in the brain of shrews after being placed in a captive environment. Such changes
266 could influence phenotype through shifts in energy metabolism, synaptic function, cell survival, and
267 behavior. Across pathways, captivity-associated changes in the shrew resemble responses in several
268 human diseases, notably, neurodegeneration. These are unlikely to be shrew-specific, as similar
269 expression shifts have been found in other organisms with disturbance in their natural environments.
270 When investigating the molecular mechanisms of disease using model organisms, captivity can confound
271 analyses of natural physiological processes. There are benefits to using captive organisms in disease
272 research, but our results indicate future studies should use natural environments to illuminate brain
273 function and responses to environmental change. These experiments could involve collaborative efforts
274 with ecologists and evolutionary biologists [80] to study natural models of brain change [21,81] that can
275 be validated in laboratory settings using cell lines. Alternatively, findings from laboratory-based disease
276 models can be paired with experimental groups in exclosures as close to wild as possible to assess the
277 impact of environmental factors on experimental outcomes.

278 ACKNOWLEDGMENTS

279 Images were created with a licensed version of BioRender.com.

280 GRANTS

281 Human Frontiers of Science Program, award: RGP0013/2019 (to Dina K. N. Dechmann, John Nieland,
282 Liliana M. Dávalos).

The Hearst Foundation, APS Hearst Summer Undergraduate Research Fellowship (to Maria Alejandra Bedoya Duque).

Stony Brook University, Presidential Innovation and Excellence award (to Liliana M. Dávalos, William R. Thomas was supported in part by award).

REFERENCES

[1] Fischer CP, Wright-Lichter J, Romero LM. Chronic stress and the introduction to captivity: How wild house sparrows (*Passer domesticus*) adjust to laboratory conditions. Gen Comp Endocrinol 2018;259:85–92. https://doi.org/10.1016/j.ygcen.2017.11.007.

[2] Fischer CP, Romero LM. Chronic captivity stress in wild animals is highly species-specific. Conserv Physiol 2018;7. https://doi.org/10.1093/conphys/coz093.

[3] Love AC, Lovern MB, DuRant SE. Captivity influences immune responses, stress endocrinology, and organ size in house sparrows (*Passer domesticus*). Gen Comp Endocrinol 2017;252:18–26. https://doi.org/10.1016/j.ygcen.2017.07.014.

[4] Seeber PA, Morrison T, Ortega A, East ML, Greenwood AD, Czirják GÁ. Immune differences in captive and free-ranging zebras (*Equus zebra* and *E. quagga*). Mamm Biol 2020;100:155–64. https://doi.org/10.1007/s42991-020-00006-0.

[5] Schulte PM. Changes in gene expression as biochemical adaptations to environmental change: A tribute to Peter Hochachka. Comp Biochem Physiol 2004;139:519–29. https://doi.org/10.1016/j.cbpc.2004.06.001.

[6] Calisi RM, Bentley GE. Lab and field experiments: Are they the same animal? Horm Behav 2009;56:1–10. https://doi.org/10.1016/j.yhbeh.2009.02.010.

[7] Dickens MJ, Earle KA, Romero LM. Initial transference of wild birds to captivity alters stress physiology. Gen Comp Endocrinol 2009;160:76–83. https://doi.org/10.1016/j.ygcen.2008.10.023.

[8] Cucchi T, Neaux D, Féral L, Goussard F, Adriensen H, Elleboudt F, et al. How domestication, feralization and experience-dependent plasticity affect brain size variation in *Sus scrofa*. R Soc Open Sci 2024;11. https://doi.org/10.1098/RSOS.240951.

[9] Lecorps B, Weary DM, von Keyserlingk MAG. Captivity-Induced Depression in Animals. Trends Cogn Sci 2021;25:539–41. https://doi.org/10.1016/J.TICS.2021.03.010.

[10] López-Maury L, Marguerat S, Bähler J. Tuning gene expression to changing environments: From rapid responses to evolutionary adaptation. Nat Rev Genet 2008;9:583–93. https://doi.org/10.1038/nrg2398.

[11] Morin M, Jönsson M, Wang CK, Craik DJ, Degnan SM, Degnan BM. Captivity induces a sweeping and sustained genomic response in a starfish. Mol Ecol 2023;32:3541–56. https://doi.org/10.1111/MEC.16947.

[12] Zhang P, Chen JS, Li QY, Sheng LX, Gao YX, Lu BZ, et al. Neuroprotectants attenuate hypobaric hypoxia-induced brain injuries in cynomolgus monkeys. Zool Res 2020;41:3. https://doi.org/10.24272/J.ISSN.2095-8137.2020.012.

[13] Yang M, Huang Y, Wu H, Li C, Ling S, Sun J, et al. Blood transcriptome analysis revealed the immune changes and immunological adaptation of wildness training giant pandas. Molecular Genetics and Genomics 2022;297:227–39. https://doi.org/10.1007/S00438-021-01841-7/METRICS.

[14] Hartstone-Rose A, Selvey H, Villari JR, Atwell M, Schmidt T. The Three-Dimensional Morphological Effects of Captivity. PLoS One 2014;9:e113437. https://doi.org/10.1371/journal.pone.0113437.

[15] Zack EH, Smith SM, Angielczyk KD. Effect of captivity on the vertebral bone microstructure of xenarthran mammals. Anat Rec 2022;305:1611–28. https://doi.org/10.1002/ar.24817.

[16] Tidière M, Gaillard JM, Berger V, Müller DWH, Lackey LB, Gimenez O, et al. Comparative analyses of longevity and senescence reveal variable survival benefits of living in zoos across mammals. Sci Rep 2016;6:1–7. https://doi.org/10.1038/srep36361.

[17] Marino L, Rose NA, Visser IN, Rally H, Ferdowsian H, Slootsky V. The harmful effects of captivity and chronic stress on the well-being of orcas (Orcinus orca). J Vet Behav 2020;35:69–82. https://doi.org/10.1016/j.jveb.2019.05.005.

[18] Quispe R, Villavicencio CP, Addis E, Wingfield JC, Vasquez RA. Seasonal variations of basal cortisol and high stress response to captivity in *Octodon degus*, a mammalian model species. Gen Comp Endocrinol 2014;197:65–72. https://doi.org/10.1016/j.ygcen.2013.12.007.

[19] Stryjek R, Parsons MH, Fendt M, Święcicki J, Bębas P. Let's get wild: A review of free-ranging rat assays as context-enriched supplements to traditional laboratory models. J Neurosci Methods 2021;362:109303. https://doi.org/10.1016/j.jneumeth.2021.109303.

[20] Baldoni C, Thomas WR, von Elverfeldt D, Reisert M, Làzaro J, Muturi M, et al. Histological and MRI brain atlas of the common shrew, *Sorex araneus*, with brain region-specific gene expression profiles. Front Neuroanat 2023;17. https://doi.org/10.3389/fnana.2023.1168523.

[21] Lázaro J, Hertel M, Sherwood CC, Muturi M, Dechmann DKN. Profound seasonal changes in brain size and architecture in the common shrew. Brain Struct Funct 2018;223:2823–40. https://doi.org/10.1007/s00429-018-1666-5.

[22] Lázaro J, Hertel M, LaPoint S, Wikelski M, Stiehler M, Dechmann DKN. Cognitive skills of common shrews (*Sorex araneus*) vary with seasonal changes in skull size and brain mass. J Exp Biol 2018;221. https://doi.org/10.1242/jeb.166595.

[23] Lázaro J, Hertel M, Muturi M, Dechmann DKN. Seasonal reversible size changes in the braincase and mass of common shrews are flexibly modified by environmental conditions. Sci Rep 2019;9. https://doi.org/10.1038/s41598-019-38884-1.

[24] Yohe LR, Davies KTJ, Rossiter SJ, Dávalos LM. Expressed Vomeronasal Type-1 Receptors (V1rs) in Bats Uncover Conserved Sequences Underlying Social Chemical Signaling. Genome Biol Evol 2019;11:2741. https://doi.org/10.1093/gbe/evz179.

[25] Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018;34:i884. https://doi.org/10.1093/bioinformatics/bty560.

[26] Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol 2016;34:525–7. https://doi.org/10.1038/nbt.3519.

[27] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:1–21. https://doi.org/10.1186/s13059-014-0550-8.

[28] Love MI, Anders S, Huber W. Analyzing RNA-seq data with DESeq2 2023. https://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#variance-stabilizing-transformation (accessed February 5, 2023).

[29] Keicher L, Teague O'mara M, Voigt CC, Dechmann DKN. Stable carbon isotopes in breath reveal fast metabolic incorporation rates & seasonally variable but rapid fat turnover in the common shrew (Sorex araneus). J Exp Biol 2017;220:2834–41. https://doi.org/10.1242/jeb.159947.

[30] Lázaro J, Dechmann DKN, LaPoint S, Wikelski M, Hertel M. Profound reversible seasonal changes of individual skull size in a mammal. Curr Biol 2017;27:R1106–7. https://doi.org/10.1016/j.cub.2017.08.055.

[31] Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Series B Stat Methodol 1995;57:289–300. https://doi.org/10.1111/J.2517-6161.1995.TB02031.X.

[32] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47. https://doi.org/10.1093/nar/gkv007.

[33] Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022;50:W216–21. https://doi.org/10.1093/nar/gkac194.

[34] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57. https://doi.org/10.1038/nprot.2008.211.

[35] Jiang H, Doerge RW. Estimating the Proportion of True Null Hypotheses for Multiple Comparisons. Cancer Inform 2008:25–32.

[36] Todd E V., Black MA, Gemmell NJ. The power and promise of RNA-seq in ecology and evolution. Mol Ecol 2016;25:1224–41. https://doi.org/10.1111/mec.13526.

[37] Benítez-Burraco A, Uriagereka J, Nataf S. The genomic landscape of mammal domestication might be orchestrated by selected transcription factors regulating brain and craniofacial development. Development Genes and Evolution 2023 233:2 2023;233:123–35. https://doi.org/10.1007/S00427-023-00709-7.

390 [38] Lu C, Li M, Sun X, Li N, Wang W, Tong P, et al. Comparing the hippocampal miRNA expression
391 profiles of wild and domesticated Chinese tree shrews (*Tupaia belangeri chinensis*). BMC Ecol
392 Evol 2021;21. https://doi.org/10.1186/S12862-020-01740-2.

393 [39] O' Regan HJ, Kitchener AC. The effects of captivity on the morphology of captive, domesticated
394 and feral mammals. vol. 35. 2005. https://doi.org/10.1111/j.1365-2907.2005.00070.x.

395 [40] Reyes AS, Bittar A, Ávila LC, Botia C, Esmeral NP, Bloch NI. Divergence in brain size and brain
396 region volumes across wild guppy populations. Proceedings of the Royal Society B 2022;289.
397 https://doi.org/10.1098/RSPB.2021.2784.

398 [41] Frahm H, Stephan H. Vergleichende Volumenmessungen an Hirnen von Wild- und
399 Gefangenschaftstieren des Spitzhornchens (*Tupaia*). Journal fuer Hirnforschung. 1976;17:449–
400 462.

401 [42] Albert FW, Somel M, Carneiro M, Aximu-Petri A, Halbwax M, Thalmann O, et al. A Comparison of
402 Brain Gene Expression Levels in Domesticated and Wild Animals. PLoS Genet 2012;8.
403 https://doi.org/10.1371/journal.pgen.1002962.

404 [43] Chadaeva I, Ponomarenko P, Kozhemyakina R, Suslov V, Bogomolov A, Klimova N, et al.
405 Domestication Explains Two-Thirds of Differential-Gene-Expression Variance between Domestic
406 and Wild Animals; The Remaining One-Third Reflects Intraspecific and Interspecific Variation.
407 Animals 2021;11:2667. https://doi.org/10.3390/ani11092667.

408 [44] Sato DX, Rafati N, Ring H, Younis S, Feng C, Blanco-Aguiar JA, et al. Brain Transcriptomics of Wild
409 and Domestic Rabbits Suggests That Changes in Dopamine Signaling and Ciliary Function
410 Contributed to Evolution of Tameness. Genome Biol Evol 2020;12:1918–28.
411 https://doi.org/10.1093/gbe/evaa158.

412 [45] DuRant S, Love AC, Belin B, Tamayo-Sanchez D, Santos Pacheco M, Dickens MJ, et al. Captivity
413 alters neuroendocrine regulators of stress and reproduction in the hypothalamus in response to
414 acute stress. Gen Comp Endocrinol 2020;295. https://doi.org/10.1016/j.ygcen.2020.113519.

415 [46] Yagound B, West AJ, Richardson MF, Gruber J, Reid JG, Whiting MJ, et al. Captivity induces large
416 and population-dependent brain transcriptomic changes in wild-caught cane toads (*Rhinella*
417 *marina*). Mol Ecol 2022:4949–61. https://doi.org/10.1111/mec.16633.

418 [47] Roznere I, Sinn BT, Daly M, Watters GT. Freshwater mussels (Unionidae) brought into captivity
419 exhibit up-regulation of genes involved in stress and energy metabolism. Sci Rep 2021;11:1–10.
420 https://doi.org/10.1038/s41598-021-81856-7.

421 [48] Maas AE, Lawson GL, Bergan AJ, Tarrant AM. Exposure to CO2 influences metabolism,
422 calcification and gene expression of the thecosome pteropod *Limacina retroversa*. J Exp Biol
423 2018;221. https://doi.org/10.1242/jeb.164400.

424 [49] Kneussel M, Wagner W. Myosin motors at neuronal synapses: Drivers of membrane transport
425 and actin dynamics. Nat Rev Neurosci 2013;14:233–47. https://doi.org/10.1038/nrn3445.

426 [50] Ma X, Kawamoto S, Uribe J, Adelstein RS. Function of the Neuron-specific Alternatively Spliced
427 Isoforms of Nonmuscle Myosin II-B during Mouse Brain Development. Mol Biol Cell
428 2006;17:2138–49. https://doi.org/10.1091/mbc.E05-10.

429 [51] Miyazaki T, Watanabe M, Yamagishi A, Takahashi M. B2 exon splicing of nonmuscle myosin heavy
430 chain IIB is differently regulated in developing and adult rat brain. Neurosci Res 2000;37.
431 https://doi.org/https://doi.org/10.1016/S0168-0102(00)00130-9.

432 [52] Rex CS, Gavin CF, Rubio MD, Kramar EA, Chen LY, Jia Y, et al. Myosin IIb Regulates actin dynamics
433 during synaptic plasticity and memory formation. Neuron 2010;67:603–17.
434 https://doi.org/10.1016/j.neuron.2010.07.016.

435 [53] Abraham G, Kowalczyk A, Loi S, Haviv I, Zobel J. Prediction of breast cancer prognosis using gene
436 set statistics provides signature stability and biological context. BMC Bioinformatics 2010;11:277.
437 https://doi.org/https://doi.org/10.1186/1471-2105-11-277.

438 [54] Hetman M, Slomnicki LP. Ribosomal biogenesis as an emerging target of neurodevelopmental
439 pathologies. J Neurochem 2019;148:325. https://doi.org/10.1111/jnc.14576.

440 [55] Kumar V. Introduction to ribosome factory, origin, and evolution of translation. In: Emerging
441 Concepts in Ribosome Structure, Biogenesis, and Function. Cambridge: Academic Press; 2021. p.
442 1–13.

443 [56] Chaillou T, Kirby TJ, Mccarthy JJ. Ribosome biogenesis: emerging evidence for a central role in the
444 regulation of skeletal muscle mass. J Cell Physiol 2014;229:1584.
445 https://doi.org/10.1002/jcp.24604.

446 [57] Donati G, Montanaro L, Derenzini M. Ribosome biogenesis and control of cell proliferation: p53 is
447 not alone. Cancer Res 2012;72:1602–7. https://doi.org/10.1158/0008-5472.can-11-3992.

448 [58] 58. Kumar V. rDNA gene structure, transcription, and its coregulation. In: Emerging Concepts in
449 Ribosome Structure, Biogenesis, and Function. Cambridge: Academic Press; 2021. p. 33–45.

450 [59] Picard M, McEwen BS. Psychological Stress and Mitochondria: A Systematic Review. Psychosom
451 Med 2018;80:141. https://doi.org/10.1097/psy.0000000000000545.

452 [60] Trabjerg MS, Mørkholt AS, Lichota J, Oklinski MKE, Andersen DC, Jønsson K, et al. Dysregulation
453 of metabolic pathways by carnitine palmitoyl-transferase 1 plays a key role in central nervous
454 system disorders: experimental evidence based on animal models. Sci Rep 2020;10:1–19.
455 https://doi.org/10.1038/S41598-020-72638-8.

456 [61] Smagin DA, Kovalenko IL, Galyamina AG, Bragin AO, Orlov YL, Kudryavtseva NN. Dysfunction in
457 Ribosomal Gene Expression in the Hypothalamus and Hippocampus following Chronic Social
458 Defeat Stress in Male Mice as Revealed by RNA-Seq. Neural Plast 2016;2016.
459 https://doi.org/10.1155/2016/3289187.

460 [62] Ansari F, Yoval-Sánchez B, Niatsetskaya Z, Sosunov S, Stepanova A, Garcia C, et al. Quantification
461 of NADH:ubiquinone oxidoreductase (complex I) content in biological samples. J Biol Chem
462 2021;297:297–301. https://doi.org/10.1016/j.jbc.2021.101204.

463 [63] Stroud DA, Surgenor EE, Formosa LE, Reljic B, Frazier AE, Dibley MG, et al. Accessory subunits are
464 integral for assembly and function of human mitochondrial complex I. Nature 2016 538:7623
465 2016;538:123–6. https://doi.org/10.1038/nature19754.

466 [64] Koopman WJH, Verkaart S, Visch HJ, Van Emst-De Vries S, Nijtmans LGJ, Smeitink JAM, et al.
467 Human NADH:ubiquinone oxidoreductase deficiency: Radical changes in mitochondrial
468 morphology? Am J Physiol Cell Physiol 2007;293:22–9.
469 https://doi.org/10.1152/ajpcell.00194.2006.

470 [65] Raffa S, Chin XLD, Stanzione R, Forte M, Bianchi F, Cotugno M, et al. The reduction of NDUFC2
471 expression is associated with mitochondrial impairment in circulating mononuclear cells of
472 patients with acute coronary syndrome. Int J Cardiol 2019;286:127–33.
473 https://doi.org/10.1016/j.ijcard.2019.02.027.

474 [66] Torraco A, Bianchi M, Verrigni D, Gelmetti V, Riley L, Niceta M, et al. A novel mutation in
475 NDUFB11 unveils a new clinical phenotype associated with lactic acidosis and sideroblastic
476 anemia. Clin Genet 2017;91:441–7. https://doi.org/10.1111/cge.12790.

477 [67] Fato R, Bergamini C, Bortolus M, Maniero AL, Leoni S, Ohnishi T, et al. Differential effects of
478 mitochondrial Complex I inhibitors on production of reactive oxygen species. Biochimica et
479 Biophysica Acta (BBA) - Bioenergetics 2009;1787:384–92.
480 https://doi.org/10.1016/j.bbabio.2008.11.003.

481 [68] Giachin G, Bouverot R, Acajjaoui S, Pantalone S, Soler-López M. Dynamics of Human
482 Mitochondrial Complex I Assembly: Implications for Neurodegenerative Diseases. Front Mol
483 Biosci 2016;3:43. https://doi.org/10.3389/fmolb.2016.00043.

484 [69] Hoefs SJG, Dieteren CEJ, Distelmaier F, Janssen RJRJ, Epplen A, Swarts HGP, et al. NDUFA2
485 Complex I Mutation Leads to Leigh Disease. Am J Hum Genet 2008;82:1306–15.
486 https://doi.org/10.1016/j.ajhg.2008.05.007.

487 [70] McElroy GS, Chakrabarty RP, D'Alessandro KB, Vasan K, Tan J, Stoolman JS, et al. Reduced
488 expression of mitochondrial complex I subunit Ndufs2 does not impact healthspan in mice. Sci
489 Rep 2022;12. https://doi.org/10.1038/S41598-022-09074-3.

490 [71] Anitha A, Nakamura K, Thanseem I, Matsuzaki H, Miyachi T, Tsujii M, et al. Downregulation of the
491 Expression of Mitochondrial Electron Transport Complex Genes in Autism Brains. Brain Pathol
492 2013;23:294. https://doi.org/10.1111/bpa.12002.

493 [72] Weydt P, Pineda V V., Torrence AE, Libby RT, Satterfield TF, Lazarowski ERR, et al.
494 Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-
495 1α in Huntington's disease neurodegeneration. Cell Metab 2006;4:349–62.
496 https://doi.org/10.1016/j.cmet.2006.10.004.

497 [73] Liang WS, Reiman EM, Valla J, Dunckley T, Beach TG, Grover A, et al. Alzheimer's disease is
498 associated with reduced expression of energy metabolism genes in posterior cingulate neurons.
499 PNAS 2008;18:4441–6. https://doi.org/10.1073pnas.0709259105.

500 [74] Chandrasekaran K, Hatanpää K, Brady DR, Rapoport SI. Evidence for Physiological Down-
501 regulation of Brain Oxidative Phosphorylation in Alzheimer's Disease. Exp Neurol 1996;142:80–8.
502 https://doi.org/10.1006/exnr.1996.0180.

503 [75] Ventura-Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, et al.
504 Mitochondria: a central target for sex differences in pathologies. Clin Sci 2017;131:803–22.
505 https://doi.org/10.1042/CS20160485.

506 [76] Gaignard P, Savouroux S, Liere P, Pianos A, Thérond P, Schumacher M, et al. Effect of Sex
507 Differences on Brain Mitochondrial Function and Its Suppression by Ovariectomy and in Aged
508 Mice. Endocrinology 2015;156:2893–904. https://doi.org/10.1210/EN.2014-1913.

509 [77] Junker A, Juster RP, Picard M. Integrating sex and gender in mitochondrial science. Curr Opin
510 Physiol 2022;26:100536. https://doi.org/10.1016/J.COPHYS.2022.100536.

511 [78] Booher WC, Vanderlinden LA, Hall LA, Thomas AL, Evans LM, Saba LM, et al. Hippocampal RNA
512 sequencing in mice selectively bred for high and low activity. Genes Brain Behav 2023;22:e12832.
513 https://doi.org/10.1111/gbb.12832.

514 [79] Misiewicz Z, Iurato S, Kulesskaya N, Salminen L, Rodrigues L, Maccarrone G, et al. Multi-omics
515 analysis identifies mitochondrial pathways associated with anxiety-related behavior. PLoS Genet
516 2019. https://doi.org/10.1371/journal.pgen.1008358.

517 [80] Bertile F, Matallana-Surget S, Tholey A, Cristobal S, Armengaud J. Diversifying the concept of
518 model organisms in the age of -omics. Commun Biol 2023;6. https://doi.org/10.1038/S42003-
519 023-05458-X.

520 [81] van Tussenbroek IA, Knörnschild M, Nagy M, Ten Cate CJ, Vernes SC. Morphological Diversity in
521 the Brains of 12 Neotropical Bat Species. Https://DoiOrg/103161/15081109ACC2023252011
522 2024;25:323–38. https://doi.org/10.3161/15081109ACC2023.25.2.011.

523

524 # FIGURE LEGENDS

525

526 **Figure 1.**

527 Differential gene expression between wild (n=4) and captive (n=4) shrews in the cortex, olfactory bulb,
528 and hippocampus. *Left:* PCA of the top 500 varying genes for each brain region. *Right*: Volcano plots for
529 the cortex, olfactory bulb, and hippocampus gene expression. Significant ($p_{adj} < 0.05$) DEGs are shown in
530 red (downregulated) or blue (upregulated) relative to wild, DEGs of interest are labeled. Labeled
531 downregulated genes encode for ribosomal proteins and mitochondrial complex I subunit proteins,
532 which have also been differentially expressed in previous studies. Labeled upregulated genes
533 correspond to the top 6 genes from the most significant upregulated enriched pathways.

534 **Figure 2.**

535 Overlapping DEGs for the three brain regions. The side histogram corresponds to the number of DEGs
536 for each brain region (set size), and the top histogram corresponds to intersections between the sets of
537 DEGs across the three brain regions. All the 104 DEGs that overlap between the three brain regions
538 (highlighted in blue) have the same directional change in gene expression (79 downregulated, 25
539 upregulated).

540 **Figure 3.**

541 *Left:* Pathway analysis for captive and wild shrews for the cortex, olfactory bulb and hippocampus. These
542 pathways were significantly enriched across the three brain regions when analyzing downregulated
543 genes, and were also enriched when analyzing only the 104 overlapping significant genes. *Right:* Side
544 histogram represents percentage of complex I subunit genes in each of these pathways.

545 TABLES

546

547 **Table 1.** – Descriptions of individuals used in gene expression experiments.

Individual	Condition	Sex	Toothrow (mm)	Cortex	Hippocampus	Olfactory Bulb
1	Wild	F	7.15	X	X	X
2	Wild	M	7.05	X	X	X
3	Wild	F	7.1	X	X	X
4	Wild	M	7.15	X	X	X
5	Captive	M	7	X	X	X
6	Captive	M	7	X	X	NA
7	Captive	M	6.9	X	X	X
8	Captive	M	7.25	X	X	X
9	Captive	M	6.9	NA	NA	X

548

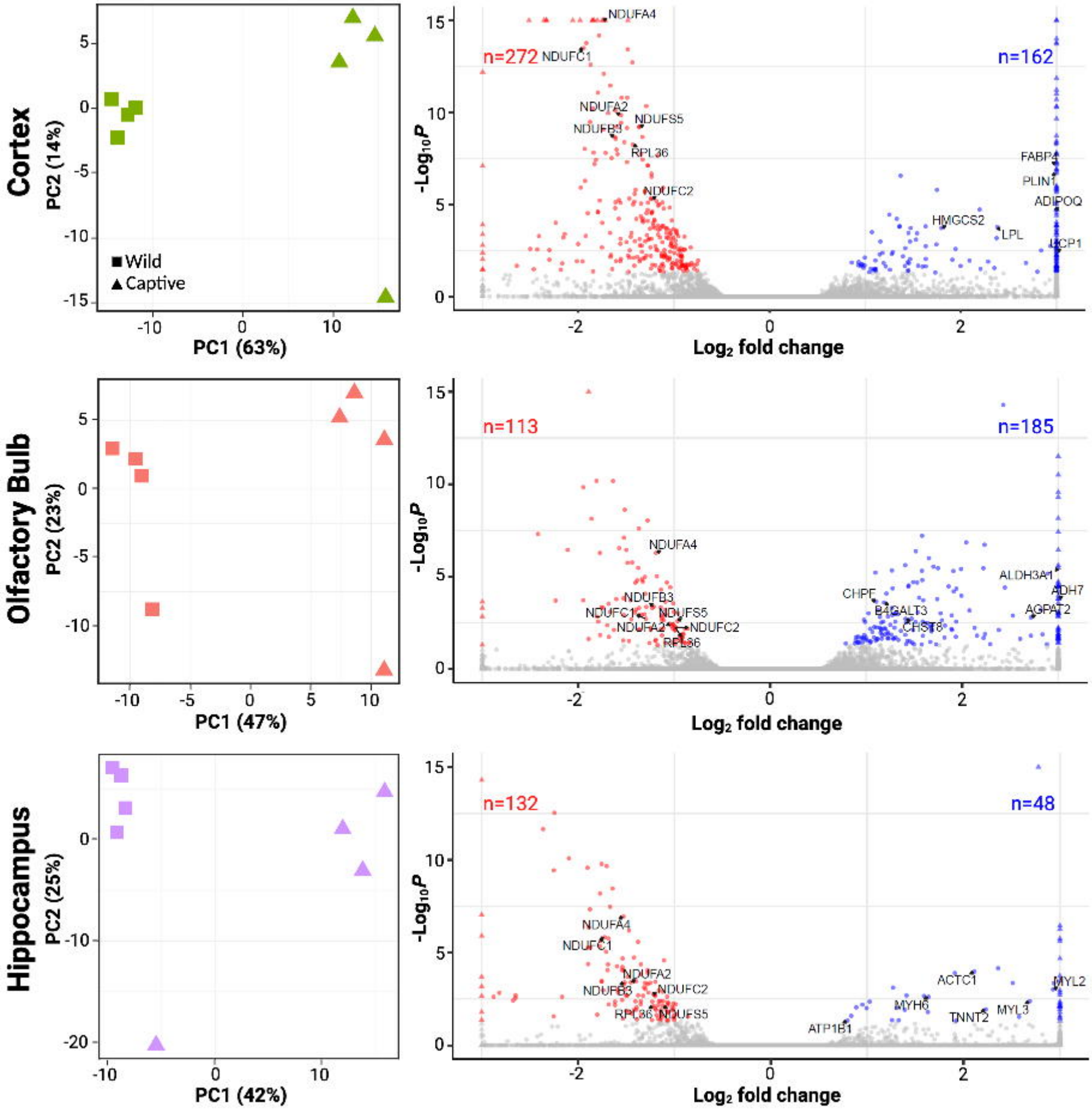
549

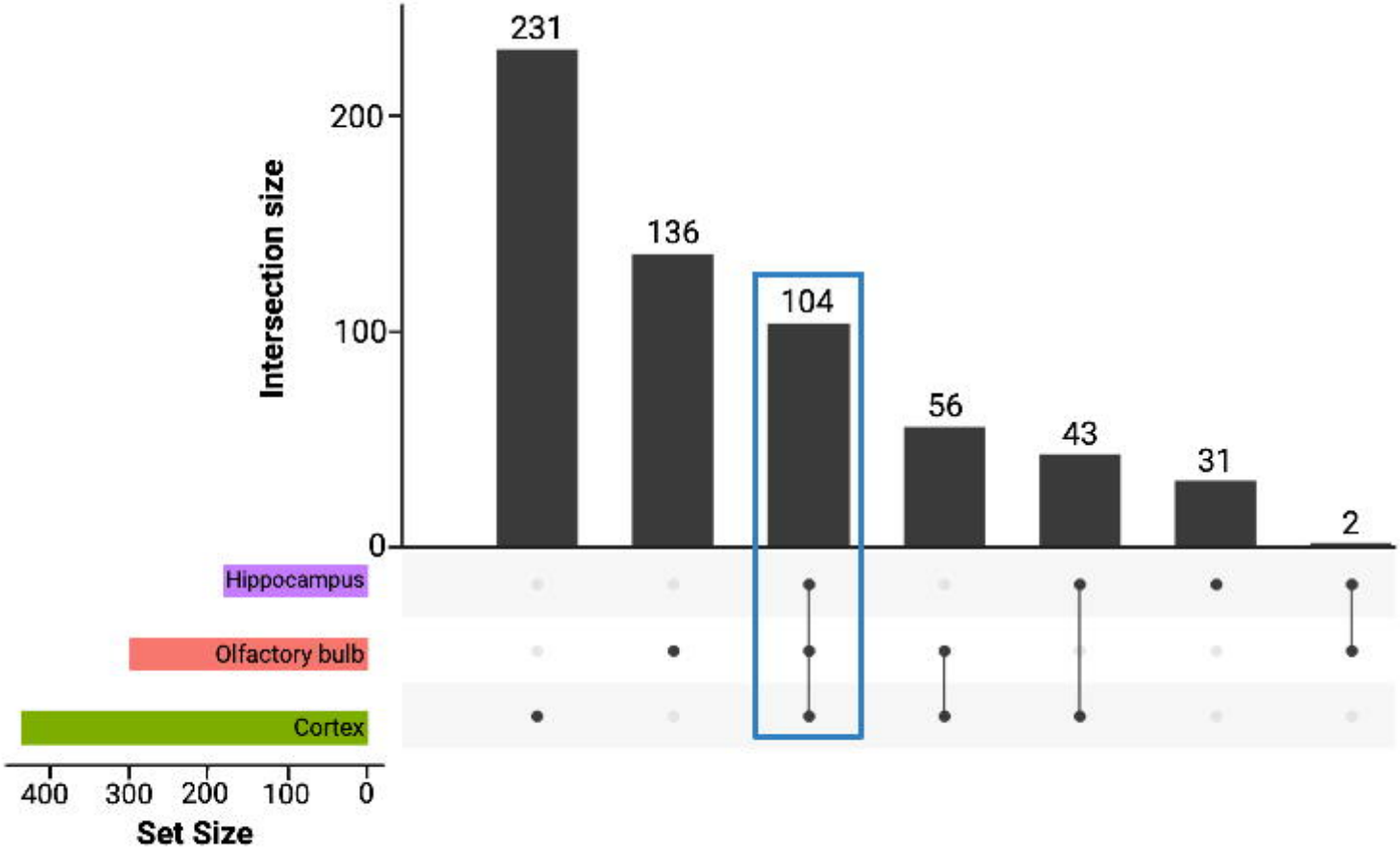
550 SUPPLEMENTAL MATERIAL

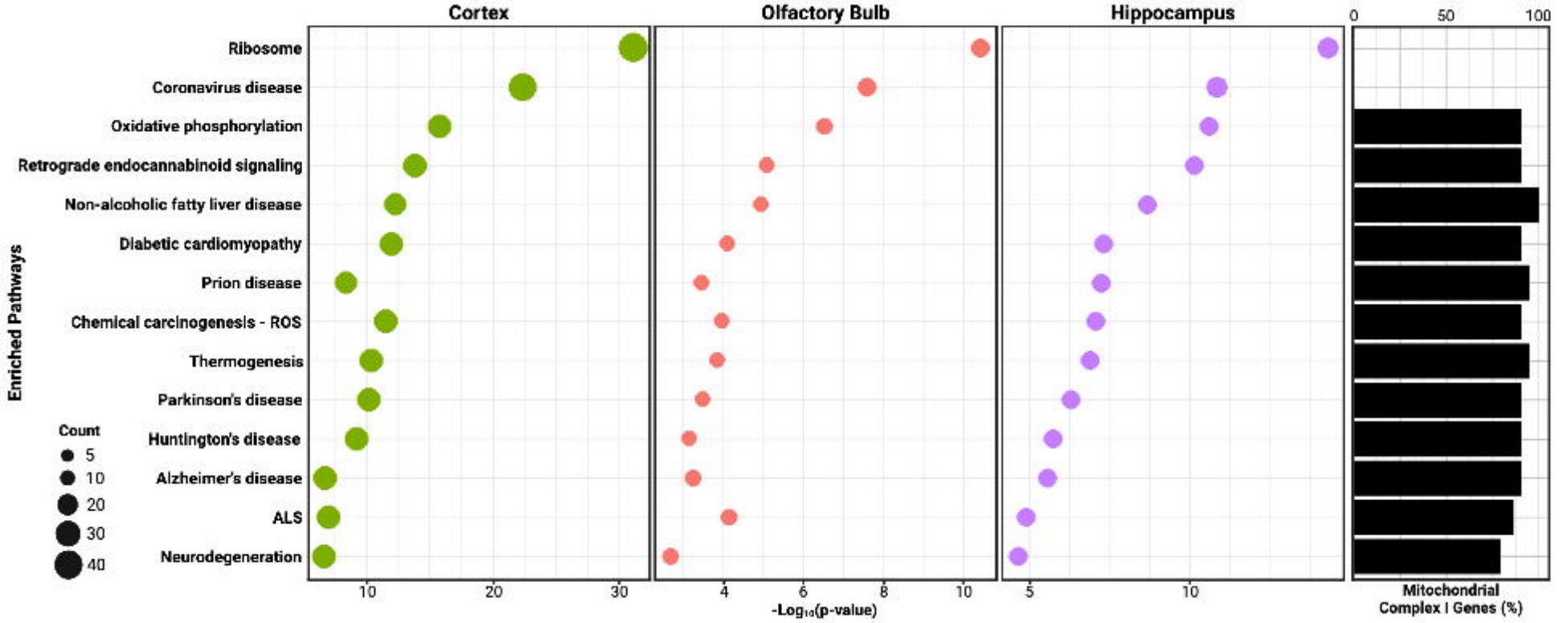
551 Supplemental Figs. S1–S7, supplemental table 2: https://doi.org/10.6084/m9.figshare.27237300.v1

552 Supplemental Table 1: DOI: https://doi.org/10.5061/dryad.qz612jmng

553


Cortex
PC2 (14%)
PC1 (63%)
Wild
Captive
n=272
n=162
NDUFA4
NDUFC1
NDUFA2
NDUFS5
NDUFB3
RPL36
NDUFC2
FABP4
PLIN1
ADIPOQ
HMGCS2
LPL
UCP1
-Log10P
Log2 fold change
Olfactory Bulb
PC2 (23%)
PC1 (47%)
n=113
n=185
NDUFA4
NDUFB3
NDUFC1
NDUFS5
NDUFA2
NDUFC2
RPL36
ALDH3A1
ADH7
CHPF
B4GALT3
AGPAT2
CHST8
Hippocampus
PC2 (25%)
PC1 (42%)
n=132
n=48
NDUFA4
NDUFC1
NDUFA2
NDUFB3
NDUFC2
RPL36
NDUFS5
ACTC1
MYL2
MYH6
TNNT2
MYL3
ATP1B1


Intersection size
200
100
0
231
136
104
56
43
31
2
Hippocampus
Olfactory bulb
Cortex
400
300
200
100
0
Set Size


Cortex
Olfactory Bulb
Hippocampus
Enriched Pathways
Ribosome
Coronavirus disease
Oxidative phosphorylation
Retrograde endocannabinoid signaling
Non-alcoholic fatty liver disease
Diabetic cardiomyopathy
Prion disease
Chemical carcinogenesis - ROS
Thermogenesis
Parkinson's disease
Huntington's disease
Alzheimer's disease
ALS
Neurodegeneration
Count
5
10
20
30
40
10
20
30
4
6
8
10
5
10
-Log10(p-value)
0
50
100
Mitochondrial Complex I Genes (%)