Supplemental information

Timed exercise stabilizes behavioral rhythms

but not molecular programs

in the brain's suprachiasmatic clock

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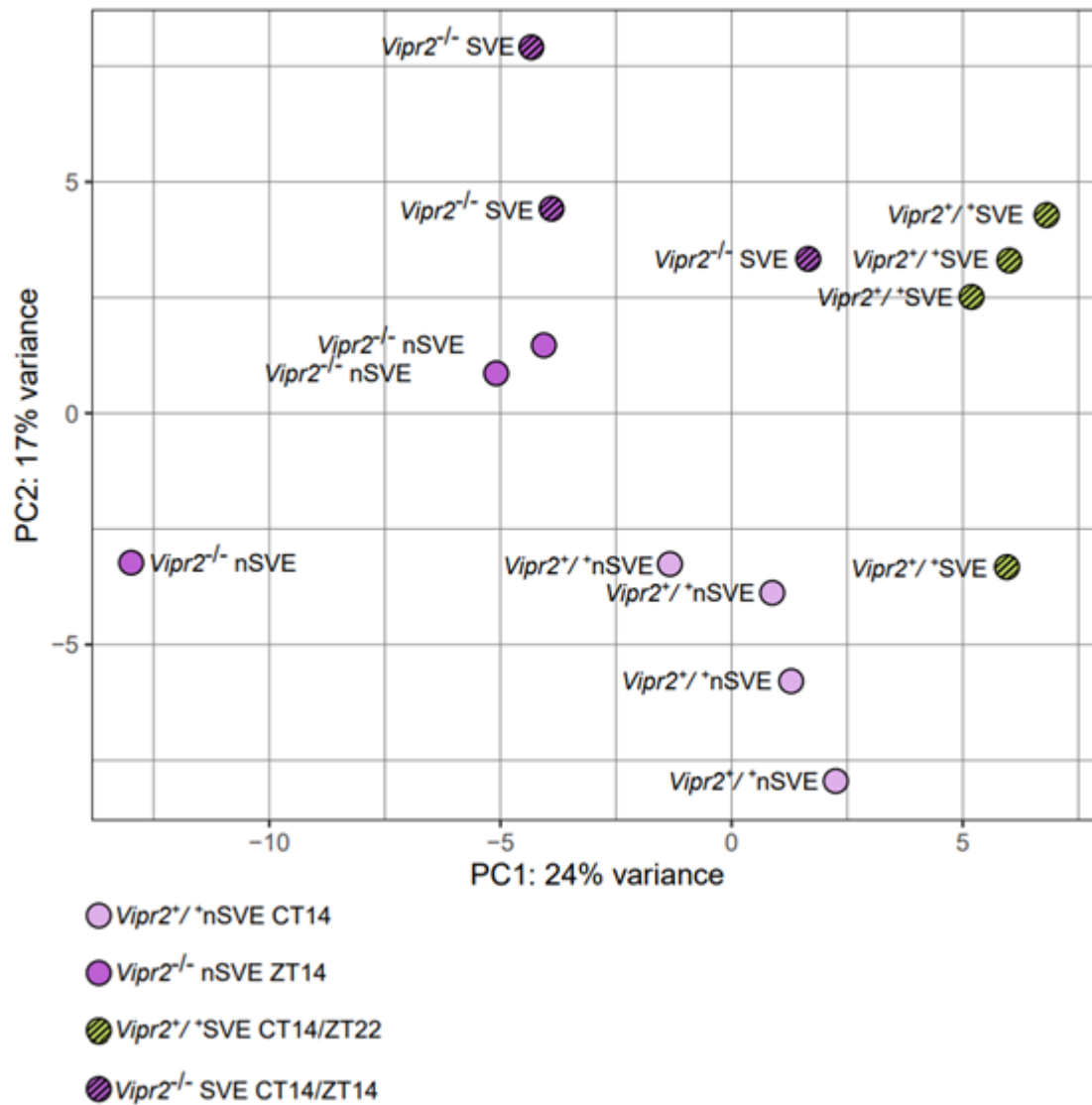
Figure S1. Principal component analysis (PCA) of RNAseq gene expression data, related to Figure 3. Most genotype and/or experimental condition samples appear to cluster. Data were analyzed by DeSeq2 method (see Methods section for further details).

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a

Vipr2⁻ᐟ⁻ vs *Vipr2⁺ᐟ⁺* nSVE

Vipr2^{-/-} vs *Vipr2^{+/+}* nSVE

KEGG pathway	Name	q-value	Gene count	Direction of regulation
mmu05034	Alcoholism	0.004740334	8	Upregulated
mmu05031	Amphetamine addiction	0.004740334	5	Upregulated
mmu04726	Serotonergic synapse	0.006340219	6	Upregulated
mmu04728	Dopaminergic synapse	0.006340219	6	Upregulated
mmu05030	Cocaine addiction	0.006340219	4	Upregulated
mmu04724	Glutamatergic synapse	0.016077478	5	Upregulated
mmu04926	Relaxin signaling pathway	0.025084251	5	Upregulated
mmu04723	Retrograde endocannabinoid signaling	0.035810389	5	Upregulated
mmu04727	GABAergic synapse	0.035810389	4	Upregulated
mmu05032	Morphine addiction	0.035810389	4	Upregulated

b

Vipr2⁺ᐟ⁺ SVE vs nSVE

KEGG pathway	Name	q-value	Gene count	Direction of regulation
mmu04360	Axon guidance	7.67408E-07	20	Upregulated
mmu04724	Glutamatergic synapse	0.117552692	9	Upregulated
mmu00515	Mannose type O-glycan biosynthesis	0.124383602	4	Upregulated
mmu04390	Hippo signaling pathway	0.124383602	10	Upregulated
mmu04929	GnRH secretion	0.124383602	6	Upregulated
mmu04260	Cardiac muscle contraction	0.124383602	7	Upregulated
mmu04727	GABAergic synapse	0.124383602	7	Upregulated
mmu04930	Type II diabetes mellitus	0.124383602	5	Upregulated
mmu04666	Fc gamma R-mediated phagocytosis	0.124383602	7	Upregulated
mmu03010	Ribosome	3.21762E-13	22	Downregulated
mmu05171	Coronavirus disease - COVID-19	1.34363E-10	22	Downregulated
mmu03060	Protein export	0.000129682	6	Downregulated
mmu04070	Phosphatidylinositol signaling system	0.002133273	8	Downregulated
mmu05016	Huntington disease	0.072831157	11	Downregulated
mmu00562	Inositol phosphate metabolism	0.072831157	5	Downregulated
mmu05017	Spinocerebellar ataxia	0.072831157	7	Downregulated
mmu05012	Parkinson disease	0.072831157	10	Downregulated
mmu05020	Prion disease	0.072831157	10	Downregulated
mmu05014	Amyotrophic lateral sclerosis	0.07895987	12	Downregulated

c

Vipr2⁻ᐟ⁻ SVE v nSVE

KEGG pathway	Name	q-value	Gene count	Direction of regulation
mmu04150	mTOR signaling pathway	0.0398956	5	Upregulated
mmu01521	EGFR tyrosine kinase inhibitor resistance	0.1113208	3	Upregulated
mmu04022	cGMP-PKG signaling pathway	0.1113208	4	Upregulated
mmu05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	0.1113208	3	Upregulated
mmu04010	MAPK signaling pathway	0.1113208	5	Upregulated
mmu04666	Fc gamma R-mediated phagocytosis	0.1113208	3	Upregulated
mmu01522	Endocrine resistance	0.1113208	3	Upregulated
mmu05231	Choline metabolism in cancer	0.1127478	3	Upregulated
mmu04625	C-type lectin receptor signaling pathway	0.1450173	3	Upregulated
mmu04919	Thyroid hormone signaling pathway	0.1496492	3	Upregulated
mmu03010	Ribosome	4.677E-06	10	Downregulated
mmu05171	Coronavirus disease - COVID-19	4.711E-05	10	Downregulated
mmu05012	Parkinson disease	0.000471	9	Downregulated
mmu05415	Diabetic cardiomyopathy	0.0005179	8	Downregulated
mmu05208	Chemical carcinogenesis - reactive oxygen species	0.0005973	8	Downregulated
mmu00190	Oxidative phosphorylation	0.001612	6	Downregulated
mmu05020	Prion disease	0.001612	8	Downregulated
mmu05016	Huntington disease	0.0031648	8	Downregulated
mmu04714	Thermogenesis	0.0031648	7	Downregulated
mmu05022	Pathways of neurodegeneration - multiple diseases	0.0111819	9	Downregulated

d

Vipr2⁻ᐟ⁻ SVE vs *Vipr2⁺ᐟ⁺* nSVE

KEGG pathway	Name	q-value	Gene count	Direction of regulation
mmu04360	Axon guidance	0.000616622	11	Upregulated
mmu04727	GABAergic synapse	0.000616622	8	Upregulated
mmu04270	Vascular smooth muscle contraction	0.001598807	9	Upregulated
mmu04723	Retrograde endocannabinoid signaling	0.001598807	9	Upregulated
mmu04012	ErbB signaling pathway	0.001598807	7	Upregulated
mmu04921	Oxytocin signaling pathway	0.00166113	9	Upregulated
mmu04912	GnRH signaling pathway	0.001676455	7	Upregulated
mmu05032	Morphine addiction	0.001676455	7	Upregulated
mmu04010	MAPK signaling pathway	0.002062915	12	Upregulated
mmu04726	Serotonergic synapse	0.002062915	8	Upregulated
mmu03010	Ribosome	9.95815E-21	30	Downregulated
mmu05171	Coronavirus disease - COVID-19	6.36224E-18	31	Downregulated
mmu05020	Prion disease	3.93115E-07	20	Downregulated
mmu05016	Huntington disease	4.17547E-07	21	Downregulated
mmu05012	Parkinson disease	1.06235E-06	19	Downregulated
mmu00190	Oxidative phosphorylation	5.86812E-06	13	Downregulated
mmu05208	Chemical carcinogenesis - reactive oxygen species	1.02868E-05	16	Downregulated
mmu05010	Alzheimer disease	1.23341E-05	21	Downregulated
mmu04714	Thermogenesis	1.37033E-05	16	Downregulated
mmu05014	Amyotrophic lateral sclerosis	2.28721E-05	20	Downregulated

Figure S2. KEGG pathways, related to Figure 3. The top 10 up-regulated and down-regulated terms in the KEGG database for functional enrichment of genes (Kyoto Encyclopedia of Genes and Genomes). In panel **a.**, no KEGG terms were significantly down-regulated.
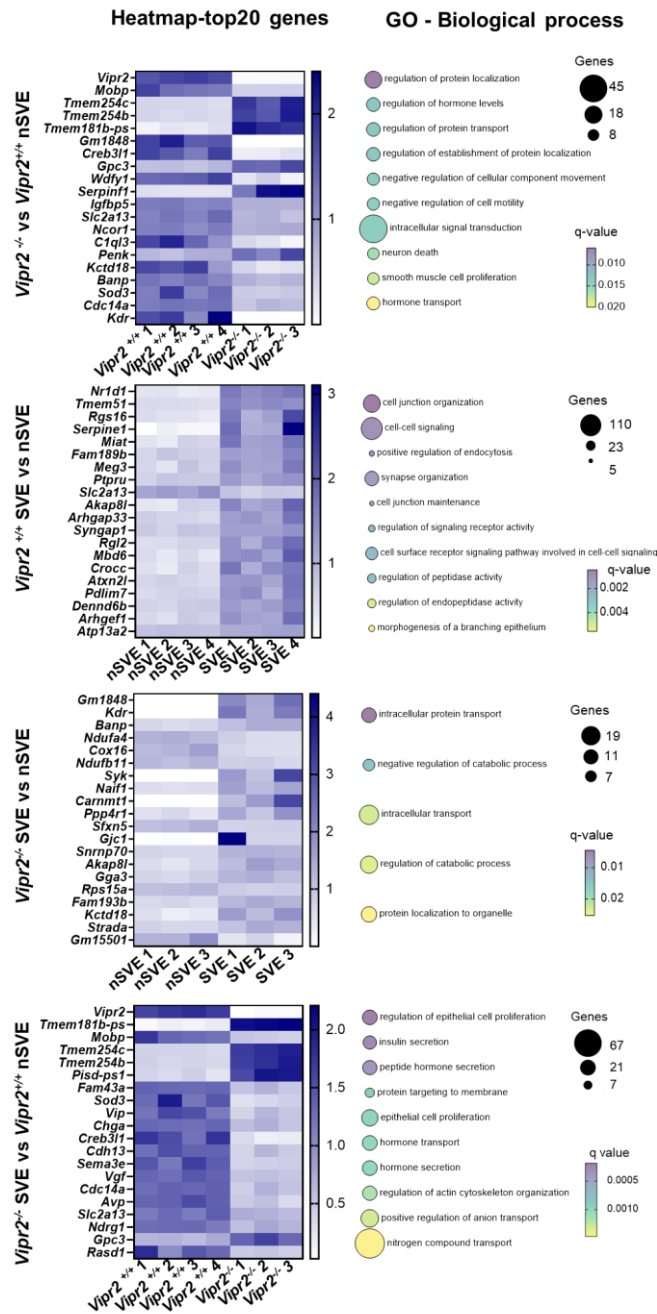
Figure S3. Top20 most differentially regulated genes and top10 most enriched Gene Ontology terms across the genotype and conditions, related to Figure 3. *Left:* Heatmap depicting the 20 most significantly differentially expressed genes in the *Vipr2^{-/-}* vs *Vipr2^{+/+}* nSVE, *Vipr2^{+/+}* SVE vs nSVE *Vipr2^{-/-}* SVE vs nSVE and *Vipr2^{-/-}* SVE vs *Vipr2^{+/+}* nSVE comparisons. The colour intensity was calculated by dividing the gene count of each individual animal with the average for the whole cohort (1=100% of mean value, the darker the colour, the more up-regulated expression in the sample). *Right:* The 10 most enriched terms in the Biological Process of the Gene Ontology Enrichment Analysis. The size of the circle is a representation of the number of genes that have been annotated, whereas the colour is indicative of the q-value.
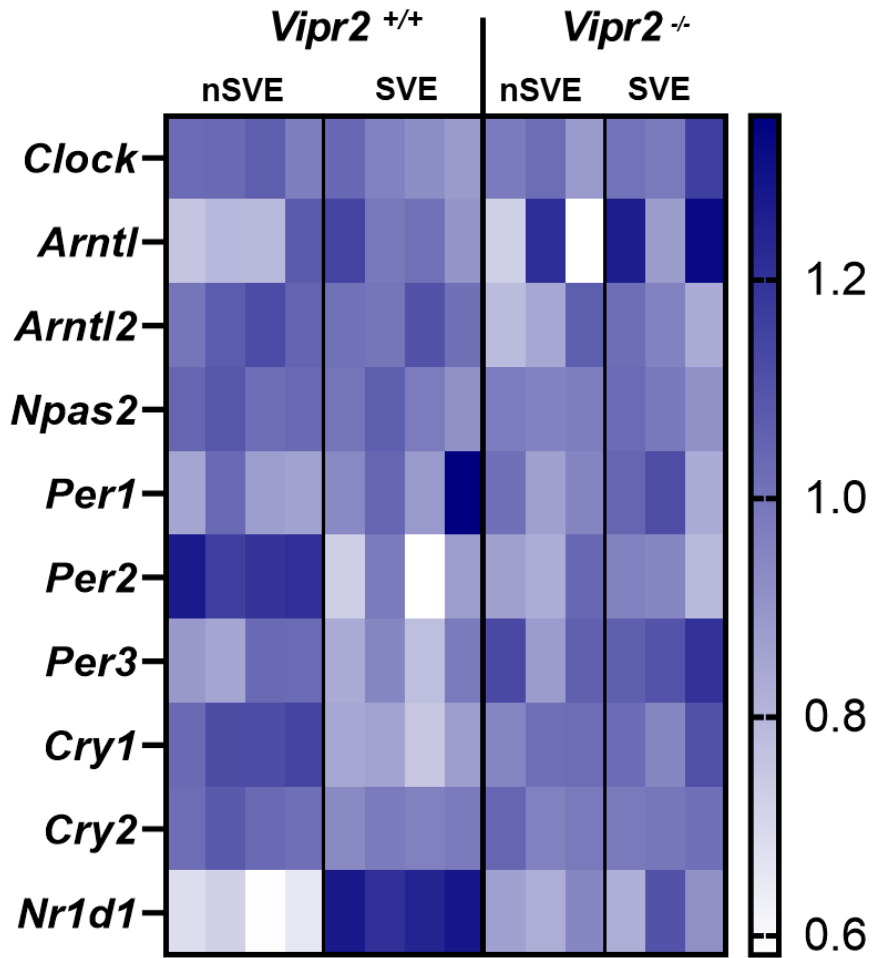
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Figure S4. Heatmap of core clock gene expression, related to Figure 4. Heatmap depicting changes in core clock gene expression quantified in RNAseq investigation in the suprachiasmatic nucleus of *Vipr2+/+* and *Vipr2-/-* mice under nSVE and SVE conditions. The gene expression for each animal was normalized to the gene's average value in the entire dataset and expressed as Log10 (with the average value=1), darker colours indicate an above average expression, whereas lighter shades correspond to below average expression.
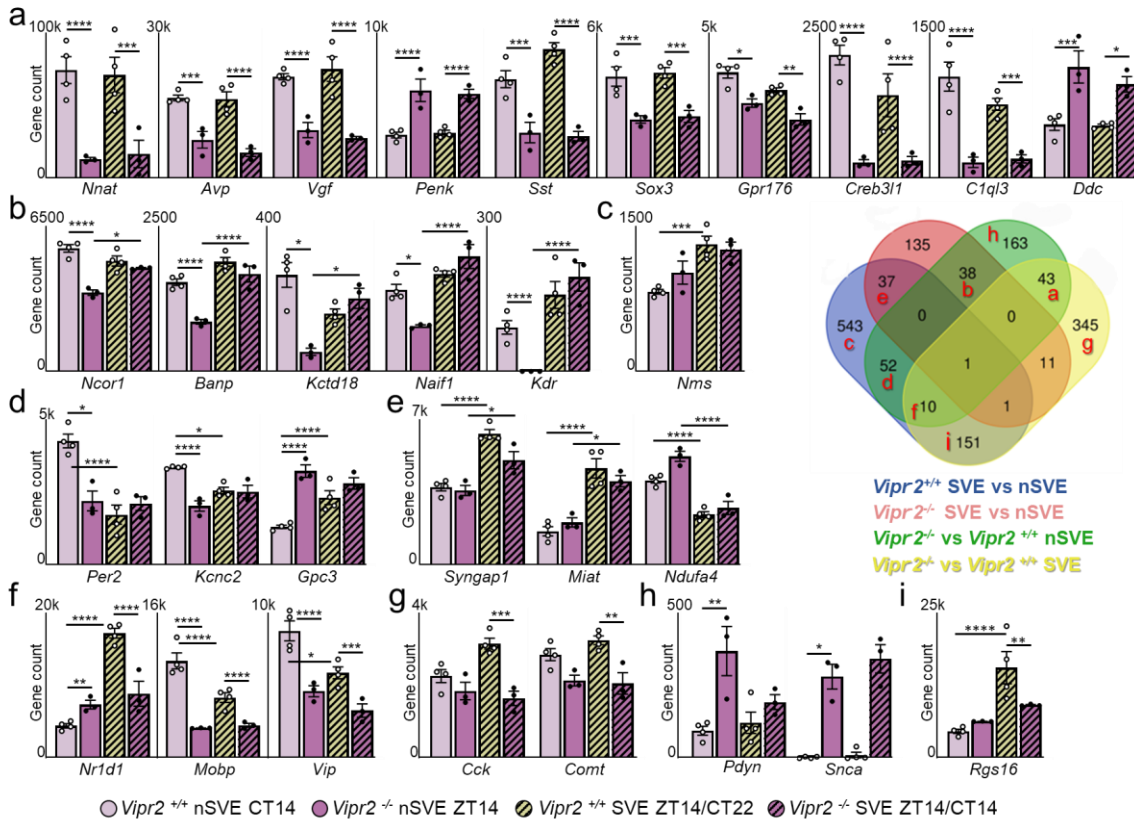
Figure S5. RNAseq gene expression counts of an expanded selection of genes that are differentially expressed across genotype and condition, related to Figure 4. Genes have been grouped by significance comparisons as illustrated in the Venn diagram on the right. For example, *Per2* is amongst the 52 genes (d) that are differentially expressed in the comparisons *Vipr2-/-* vs *Vipr2+/+* nSVE and *Vipr2+/+* SVE vs nSVE (intersection between the yellow and blue sets). Points show each animal's gene expression count with bars denoting mean value± SEM. (q value <0.05 = *, <0.01 = **, <0.005 = ***, <0.001 = ****).
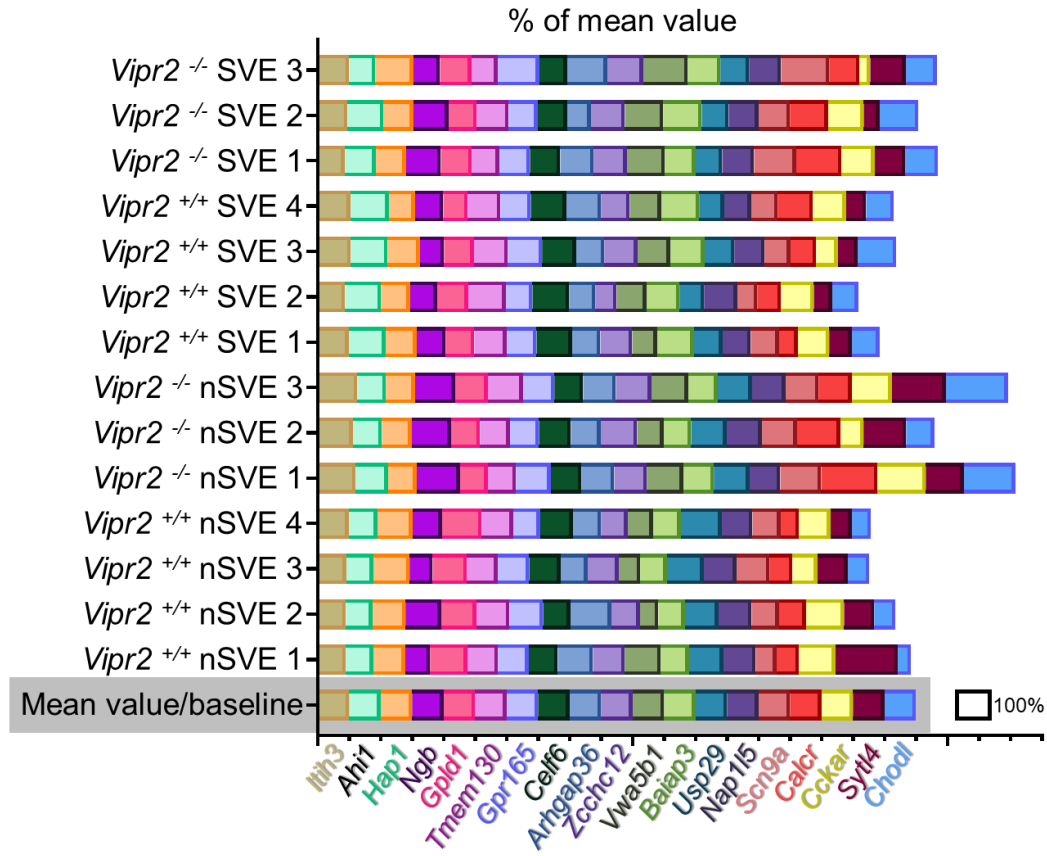
Figure S6. RNAseq gene expression counts of genes known to be enriched in the SCN across the animals, related to Figure 4. RNAseq gene counts were normalised using the built-in normalisation method in DeSeq2. A quantification of the genes described in the meta-analysis of Brown et al.[127], except for RP24-361E14.1, is shown. All genes were found to be expressed in each sample, suggesting that the SCN was sampled consistent with other studies. The values are expressed as percentage of the mean value across all samples.