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Exploratory Analysis of Sleep Deprivation Effects on Gene Expression and Regional Brain Metabolism

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Keywords

Psychomotor Vigilance · Stanford Sleepiness Scale · Affymetrix microarray · Fluorodeoxyglucose-18 positron emission tomography

Abstract

Introduction: Sleep deprivation affects cognitive performance and immune function, yet its mechanisms and biomarkers remain unclear. This study explored the relationships among gene expression, brain metabolism, sleep deprivation, and sex differences. **Methods:** Fluorodeoxyglucose-18 positron emission tomography measured brain metabolism in regions of interest, and RNA analysis of blood samples assessed gene expression pre- and post-sleep deprivation. Mixed model regression and principal component analysis identified significant genes and regional metabolic changes. **Results:** There were 23 and 28 differentially expressed probe sets for the main effects of sex and sleep deprivation, respectively, and 55 probe sets for their interaction (FDR-corrected $p < 0.05$). Functional analysis of genes affected by sleep deprivation revealed pathway enrichment in nucleoplasm- and UBL conjugation-related genes. Genes with significant sex effects mapped to chromosomes Y and 19 (Benjamini-Hochberg FDR $p < 0.05$),

with 11 genes (4%) and 29 genes (10.5%) involved, respectively. Differential gene expression highlighted sex-based differences in innate and adaptive immunity. For brain metabolism, sleep deprivation resulted in significant decreases in the left insula, left medial prefrontal cortex (BA32), left somatosensory cortex (BA1/2), and left motor premotor cortex (BA6) and increases in the right inferior longitudinal fasciculus, right primary visual cortex (BA17), right amygdala, left cerebellum, and bilateral pons. **Conclusion:** Sleep deprivation broadly impacts brain metabolism, gene expression, and immune function, revealing cellular stress responses and hemispheric vulnerability. These findings enhance our understanding of the molecular and functional effects of sleep deprivation.

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Introduction

Sleep disruptions can be caused by a wide array of factors and stressors and affect large portions of the population in modern times [1]. Most individuals

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experiencing sleep deficits and disturbances exhibit deficits in neurobehavioral functions, including cognitive speed and working memory, vigilant attention, and executive functions [2, 3]. Furthermore, sleep deprivation (SD) has been shown to elicit a significant reorganization of regional cerebral metabolic activity [4]. Over recent years, there has been an effort to explore gene expression changes associated with SD [5, 6], with numerous studies in animals examining genes whose expression in specific brain regions varies between sleep, wakefulness, and SD [7–11]. Though it is currently not feasible to directly measure gene expression in the cerebral cortexes of humans in real time, gene expression measured from blood samples may provide us with a privileged view into neurological effects associated with SD as it has been previously used to identify biomarkers for mood disorders [12–14]. Furthermore, other studies aimed at identifying molecular biomarkers in conjunction with behavioral measures have yielded potential gene expression biomarkers for SD-related cognitive impairments [15], with one study finding that individuals resistant to the behavioral effects of SD show reduced circadian rhythmicity in gene expression measured from blood samples [16]. Furthermore, there has been a recent effort to identify candidate biomarkers associated with structural and functional changes in the brains of patients with obstructive sleep apnea [17].

Despite these efforts, research that concurrently explores the cognitive and molecular effects of SD alongside regional brain metabolism in humans remains limited. This study aimed to bridge this gap by integrating gene expression analysis, cognitive performance measures, and regional brain metabolism. Through this comprehensive approach, we seek to delineate the intricate relationships among these dimensions, enhancing the understanding of how SD affects the human brain on multiple levels. Such insights could lead to more targeted and effective treatments for sleep-related disorders, thereby improving outcomes for affected individuals.

Microarray chips were utilized to assess peripheral gene expression, leveraging their high-throughput capability. This approach aligns with numerous gene expression profiling studies that employ microarray data to identify differentially expressed transcripts in subjects under specific conditions [6]. The Psychomotor Vigilance Test (PVT) – known for its high sensitivity to SD [18] – was used to gauge cognitive performance. Additionally, the Stanford Sleepiness Scale (SSS), a widely used tool in research settings to measure subjective sleepiness [19], was used as an additional measure of cognitive changes. Both PVT and SSS were utilized due to findings

that subjective measures of sleepiness do not correlate with performance on PVT following SD [2], highlighting the importance of using both objective and subjective assessment tools to gain a comprehensive understanding of the impact of SD. Furthermore, fluorodeoxyglucose (FDG)-positron emission tomography (PET) imaging was used to analyze regional brain metabolism. The multimodal data were then analyzed using principal component analysis (PCA) to characterize the variance observed across subjects.

PCA is a technique used to reduce the dimensionality of large datasets by eliminating correlated and redundant features [20, 21], by extracting new orthogonal variables called principal components. The principal components are linear combinations of the original variables and are ordered by the amount of variance they capture from the original data [21]. PCA has long been valuable for analyzing transcriptomic data [22]. More recently, its application has broadened to encompass the study of diverse diseases [23, 24] and the gene expression profiling of healthy individuals [25]. Additionally, PCA has been used to analyze fluorodeoxyglucose positron emission tomography (F18-FDG PET) brain scans in the study of aging [26] and various diseases such as Alzheimer's [27, 28] and alcohol-related cognitive impairment [29]. Due to the nature of the high-dimensional data associated with this study, we used PCA to integrate the analysis of gene expression and regional brain metabolism [30].

Methods

Sleep Deprivation

After IRB approval, a pool of potential subjects from the University of California Irvine was recruited and screened for health. Only subjects who did not have a history of eating, medical, mental health, or sleep disorders could participate in the study. Eight healthy subjects, four males and four females, were selected from the previously screened batch to participate in this study. Ethnicity data were not formally recorded for participants. On day 1, subjects were initially assigned 24 h of normal activity (e.g., walking, talking, studying, watching TV, playing games, using the computer, etc.). The subjects were tested on the Psychomotor Vigilance Test (PVT) and asked to rate their subjective level of sleepiness on the Stanford Sleepiness Scale (SSS) at baseline. The PVT is a widely used psychometric assessment of sustained attention and reaction time. Participants must respond as quickly as possible to visual or auditory stimuli that appear at random intervals on a screen, typically

Table 1. The Stanford Sleepiness Scale

Degree of sleepiness	Scale rating
Feeling active, vital, alert, or wide awake	1
Functioning at high levels but not at peak; able to concentrate	2
Awake but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy; losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep; prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

Source: Shahid et al. [78].

using a response button. Reaction times, lapses (responses >500 ms), false alarms, and omissions are recorded as key performance metrics. PVT administration for this study lasted 10 min in a quiet, controlled environment to minimize external distractions. Higher scores indicate a longer, more delayed, response time on the PVT, while higher scores on the SSS indicate greater degrees of sleepiness. The SSS is shown in Table 1. Each subject's performance on the Psychomotor Vigilance Test (PVT) and subjective sleepiness ratings (SSS) were recorded both before and after SD (Table 2). There was no significant difference in age between male and female subjects (Table 3), all of whom had no prior psychiatric history.

To assess the effects of SD on regional brain metabolism, F18-FDG PET scans were conducted at two time points: baseline (day 1, 1:00 p.m.) and post-SD (day 2, 11:00 a.m.) (Table 4). During each scan, subjects were administered F18-FDG and instructed to rest quietly with minimal sensory stimulation to ensure consistent metabolic conditions. Imaging was performed using a standardized protocol, with PET scans acquired approximately 45–60 min post-injection.

Blood samples were collected on baseline day at 1:00 p.m., pre-SD. SD activities and blood sample acquisition times are recorded in Table 4. At the end of day 1 (11:00 p.m.), subjects were moved to an outpatient research facility for the SD protocol. They were requested not to nap or sleep during the SD period and were additionally tasked with filling out forms and answering questions about their mood every 2 to 4 h. Staff members monitored the subjects during the SD period. Subjects were allowed to walk, talk, study, watch TV, play games or cards, read,

Table 2. Subject response data

Subject	Condition	Sex	Age	SSS	PVT
1	Pre-SD	F	21	2	254.56
1	Post-SD	F	21	4	339.67
2	Pre-SD	F	21	1	316.09
2	Post-SD	F	21	3	423.33
3	Pre-SD	F	19	2	358.82
3	Post-SD	F	19	3	640.13
4	Pre-SD	F	23	2	491.67
4	Post-SD	F	23	4	321.15
5	Pre-SD	M	19	1	288.09
5	Post-SD	M	19	4	338.99
6	Pre-SD	M	27	2	246.7
6	Post-SD	M	27	3	261.96
7	Pre-SD	M	34	1	250.11
7	Post-SD	M	34	2	276.48
8	Pre-SD	M	21	3	249.67
8	Post-SD	M	21	4	267.14

SD, sleep deprivation; SSS, Stanford Sleepiness Scale; PVT, Psychomotor Vigilance Task. Paired *T* test (two tailed). SSS: *t* statistic = −6.1775, *p* value = 0.0005. PVT: *t* statistic = −1.1682, *p* value = 0.2810.

and use the computer but were not allowed caffeinated foods or beverages. A second blood sample was collected 18 h after starting SD activities (SD day 2, 1:00 p.m.); subjects completed the protocol and were driven home by cab.

Table 3. Welch *T* test on age differences between sexes

Age	<i>N</i>	Mean	St dev	SEM	df	<i>t</i>	<i>p</i> value
Male	4	25.25	6.75	3.38	3	1.22	0.30
Female	4	21	1.63	0.82			

Gene Data Processing

Blood samples (3 mL) were drawn from each subject, into Tempus tubes (ABI, ThermoFisher, Carlsbad, CA, USA) 24 h apart. The blood samples collected at baseline and 18 h after starting SD activities were processed with Affymetrix HG-U133 Plus 2.0 gene expression microarray chips according to the manufacturer’s instructions (Affymetrix, ThermoFisher, Carlsbad, CA, USA). Data processing was done using R 4.2 and BioConductor 3.16 [31]. The Affymetrix HG-U133 Plus 2.0 microarray “cel” files were read using the affy routine with the hgu133plus2.db package. Quantile normalization was used to standardize probe set data [32]. A linear model was fitted to the expression data for each probe set using “lmfit” from the limma package, to eliminate weakly expressed probe sets, and the top 40,000 probe sets were found using the topTables function. Type III two-way mixed ANOVA was implemented on probe set expression data using the lmerTest library in R, with the main effects being sex, SD, and SD-sex interaction. Age and RNA integrity number were used as covariates. The top 300 probe sets for each main effect from mixed ANOVA and PCA were analyzed for enrichment using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [33, 34]. PCA was conducted using the pca function with normalized and scaled expression data.

F18-FDG PET Scan Processing

The pre-SD and post-SD F18-FDG PET scans were obtained from each subject. Each F18-FDG PET scan was normalized in MATLAB (Mathworks, Sherborn, MA, USA) using Statistical Parametric Mapping 5 software (Functional Imaging Laboratory, Wellcome Department of Cognitive Neurology, University College London, London, UK) to spatially transform the images to a template conforming to the space derived from standard brains from the Montreal Neurological Institute and convert it to the space of the stereotactic atlas of Talairach and Tournoux. Regional metabolism for each subject was normalized using subject’s intracranial volume metabolic average, with age as a covariate during normalization. The images were then smoothed with a Gaussian low-pass filter of 8 mm to minimize noise and improve spatial alignment.

Regions of interest (ROIs) analysis was performed by extracting metabolic values from ROI using “Volume Imaging in Neurological Research, Co-Registration and ROI included” software. Online supplementary Figure 1 (for all online suppl. material, see <https://doi.org/10.1159/000545461>) shows the ROI segmentation of FDG-PET scans labeled with brain regions and Brodmann areas.

A type III mixed two-way ANOVA was implemented on extracted ROI metabolic values using the lmerTest library in R. The model considered sex as a between-subjects factor and condition (pre-SD vs. post-SD) as a within-subjects factor. PCA on ROI was performed using the pca() function in the Bioconductor environment [31] in R.

Results

Two-Way Mixed ANOVA for Gene Expression

Sex

Benjamini-Hochberg (BH) correction revealed 23 probe sets reflecting 11 unique genes that had a significant sex effect in expression levels (FDR-adjusted $p < 0.05$). Table 5 shows all significant results from the gene expression mixed ANOVA (FDR-adjusted $p < 0.001$), with the strongest sex differences found in three sex chromosome genes (XIST, EIF1AY, and RPS4Y1). The functional cluster nucleoplasm was significantly enriched (FDR $p = 2.30E-04$; online suppl. Table 1). Complete mixed ANOVA results for all probe sets are provided in online supplementary Table 2, while FDR-adjusted significant probe sets are presented in Table 5 and online supplementary Table 3.

Sleep Deprivation

BH-corrected mixed ANOVA results revealed 28 probe sets reflecting 27 unique genes that showed a significant SD effect in their expression levels (Table 5; FDR-adjusted $p < 0.05$), with VAPB (adjusted $p < 0.001$) and GSDMB (adjusted $p < 0.001$) having the most significant results. A significant functional cluster for ubiquitin-like (UBL) conjugation was observed (FDR $p = 6.00E-04$; online suppl. Table 1). In total, 111 probe sets (65 genes) related to UBL conjugation and downstream targets were nominally altered as an effect of SD, with a balance of up- and downregulated gene expression patterns. The mechanistic UBL conjugation genes dysregulated by SD were ITCH, OTULIN, RNF40, TRIM33, TRIM52, and WASHC1. The remainder of the UBL conjugation cluster comprised downstream target genes (online suppl. Table 4) that drive diverse pathways. No overlap was observed between the 23 differentially expressed probe sets for sex and the 28 differentially expressed probe sets for SD.

Table 4. Sleep deprivation schedule

Time	Activity	Blood draw
<i>SD day 1</i>		
12:00 p.m.	Intake for normal control subject to BIC for SD	
1:00 p.m.	Draw blood, complete mood and cognitive ratings, take cognitive test battery, 1st PET scan	*
7:00 p.m.	Complete PVT, mood, and cognitive ratings	
11:00 p.m.	Complete mood and cognitive ratings	
<i>SD day 2</i>		
1:00 a.m.	Complete mood and cognitive ratings	
3:00 a.m.	Complete mood and cognitive ratings	
7:00 a.m.	Complete PVT, complete mood and cognitive ratings	
11:00 a.m.	Complete mood and cognitive ratings, post-SD PET scan (2nd PET scan)	
1:00 p.m.	Draw blood, complete mood and cognitive ratings, take cognitive test battery	*
3:00 p.m.	Complete mood and cognitive ratings	
7:00 p.m.	Complete PVT, mood, and cognitive ratings	
8:00 p.m.	Release subject	

Blood draw is indicated with an asterisk (*). The PVT and SSS assessments conducted at 1:00 p.m. on day 1 served as the pre-SD baseline measures, while the PVT and SSS assessments at 7:00 p.m. on day 2 were used as the post-SD measures. SD, sleep deprivation; SSS, Stanford Sleepiness Scale; PVT, Psychomotor Vigilance Task; BIC, UC Irvine Brain Imaging Center.

Interaction of Sex and SD
Fifty-five probes reflecting 55 unique genes were found to show a significant interaction of SD and sex effect (Table 5; FDR-adjusted $p < 0.05$), with PCAT7, PEX16, and TMEM11 having the most significant results (FDR $p < 0.001$). No significant functional cluster enrichment surviving FDR correction was found for the interaction effect.

PCA for Gene Expression
Variance proportions, along with cumulative variance, are illustrated in Figure 1. The first 8 principal components captured 84% of the variance in gene expression across subject samples and appeared to plateau (Fig. 1). PCA of gene expression showed that PC3 and PC4 exhibit clustering by sex (Fig. 2), suggesting that sex-related differences in gene expression contribute to observed metabolic and behavioral effects. This further supports the importance of examining the interaction between SD and sex at both the transcriptomic and neuroimaging levels.

Functional Enrichment and Pathway Analysis of Gene Principal Components
The full functional clustering analysis results from DAVID (top 300 features for each principal component used for gene functional cluster enrichment analysis) are

shown in online supplementary Table 5. The selection of the top 300 features was based on the need to retain a substantial proportion of total variance, ensuring the inclusion of biologically relevant signals while minimizing the contribution of noise from lower-variance principal components. The inclusion of an excessive number of features could introduce spurious variability, potentially diluting meaningful biological effects, whereas selecting too few features risks excluding key dimensions of gene expression variability. Additionally, the choice of 300 features allows for a sufficient dataset for robust functional enrichment analysis, facilitating the identification of biologically relevant pathways and gene clusters. The first principal component (PC1_gene) has a significant functional cluster enrichment score of 67.36 for ribosomal functions and protein synthesis within the cytoplasm. The large and small ribosomal units and processome are enriched, indicating involvement in the production and processing of the ribosomal small subunit, a crucial step in ribosome assembly. The second principal component (PC2_gene) has significant functional clusters relating to cell cycle, mitosis, chromosome segregation at the kinetochore, adaptive immunity, and biological rhythms. PC5_gene has significant functional clusters for mRNA processing and zinc finger domains.

Table 5. Gene expression mixed two-way ANOVA significant results

Gene symbol	Probe set	F value	Mean (female)	Mean (male)	BH <i>p</i> value	% fold change
Sex effect						
XIST	224590_at	425.61	3.005	−3.352	3.88E−06	−190
EIF1AY	204409_s_at	285.81	−3.576	2.705	1.97E−05	−232
XIST	214218_s_at	264.01	2.110	−2.941	2.07E−05	−172
RPS4Y1	201909_at	235.66	0.359	4.991	2.98E−05	−93
XIST	227671_at	207.59	3.409	−1.500	4.92E−05	−327
DDX3Y	205001_s_at	195.43	−0.915	0.359	5.78E−05	−355
XIST	221728_x_at	174.65	2.597	−1.571	9.28E−05	−265
EIF1AY	204410_at	170.82	−2.247	1.813	9.28E−05	−224
DDX3Y	205000_at	167.12	−2.695	2.274	9.34E−05	−219
XIST	224588_at	161.72	4.173	−1.155	1.01E−04	−461
USP9Y	228492_at	149.88	−2.691	0.629	1.32E−04	−528
PRKY	206279_at	149.30	−0.861	0.404	1.32E−04	−313
ZFY	230760_at	111.56	−3.469	−0.828	6.08E−04	319
TXLNGY	232618_at	103.20	−3.370	−0.405	8.33E−04	731
TXLNGY	223646_s_at	102.55	−0.960	0.710	8.33E−04	−235
USP9Y	206624_at	101.06	−2.840	−0.088	8.46E−04	3,129
KDM5D	206700_s_at	90.90	−0.344	2.083	1.41E−03	−117
UTY	210322_x_at	89.75	−0.255	0.449	1.42E−03	−157
TXLNGY	214131_at	80.36	−2.657	−0.427	2.42E−03	522
XIST	224589_at	64.85	0.920	−2.673	7.03E−03	−134
TXLNGY	236694_at	63.16	−2.433	−0.339	7.66E−03	618
UTY	211149_at	60.55	−3.047	−1.465	9.07E−03	108
CTSW	214450_at	53.41	2.838	3.277	1.63E−02	−13
Gene symbol	Probe set	F value	Mean pre-SD	Mean post-SD	BH <i>p</i> value	% fold change
SD effect						
VAPB	225923_at	191.50	0.147	0.379	3.89E−04	157
GSDMB	215659_at	154.45	0.742	0.370	6.54E−04	−50
MED13	244611_at	91.68	0.024	−0.344	7.62E−03	−1,535
PTPRN2	203029_s_at	79.99	0.874	0.701	9.60E−03	−20
ARSD	223696_at	79.74	0.713	0.904	9.60E−03	27
WASHC1	236841_at	75.47	2.034	1.622	1.07E−02	−20
OGFOD3	64438_at	60.40	0.269	0.447	2.58E−02	66
TLK1	220702_at	59.49	−0.506	−0.711	2.58E−02	40
GOPC	236862_at	57.37	−1.499	−1.664	2.58E−02	11
PTGS1	238669_at	56.86	2.454	2.126	2.58E−02	−13
H2BC12	209806_at	55.81	3.951	3.724	2.58E−02	−6
LTBP1	202729_s_at	55.51	1.338	1.178	2.58E−02	−12

Table 5 (continued)

Gene symbol	Probe set	<i>F</i> value	Mean pre-SD	Mean post-SD	BH <i>p</i> value	% fold change	
TAL1	206283_s_at	53.66	0.821	0.489	2.72E-02	-40	
BICD2	212702_s_at	53.23	1.925	1.637	2.72E-02	-15	
TMEM63A	214833_at	51.68	1.639	1.303	2.83E-02	-21	
CISH	223377_x_at	51.40	2.570	2.859	2.83E-02	11	
EXOSC1	1559044_at	50.44	-2.297	-2.564	2.93E-02	12	
SPAG9	212470_at	48.42	2.253	1.934	3.06E-02	-14	
TTC14	225178_at	48.24	0.959	0.761	3.06E-02	-21	
ARHGEF7	1562270_at	47.91	-2.777	-2.937	3.06E-02	6	
RUBCNL	44790_s_at	47.88	2.012	1.849	3.06E-02	-8	
IFIT2	226757_at	47.34	3.662	3.311	3.08E-02	-10	
FAM174A	226752_at	46.92	2.037	1.591	3.08E-02	-22	
PPBP	214146_s_at	46.01	6.039	5.743	3.25E-02	-5	
MALAT1	224568_x_at	44.47	4.780	3.768	3.68E-02	-21	
RUFY2	233192_s_at	43.15	-1.549	-1.663	4.09E-02	7	
MALAT1	223940_x_at	41.37	5.173	4.352	4.81E-02	-16	
DAGLB	225828_at	40.74	-0.190	-0.154	4.99E-02	-19	
Gene symbol	Probe set	<i>F</i> value	Mean change		BH <i>p</i> value	% fold change	
			female	male		female	male

Interaction effect

PCAT7	1559861_at	246.86	0.02	0.01	9.14E-05	31	8
PEX16	221604_s_at	210.34	0.02	-0.01	1.03E-04	43	-7
TMEM11	203437_at	199.58	0.00	-0.02	1.03E-04	7	-30
DAGLB	225828_at	83.37	-0.01	0.03	9.48E-03	-5	25
FLT4	210316_at	74.67	0.11	0.02	1.35E-02	169	13
LINC00242	1561327_at	70.23	0.00	0.02	1.41E-02	2	27
DPF3	238532_at	67.58	0.03	-0.02	1.41E-02	21	-10
LTBP1	202729_s_at	67.45	0.00	-0.03	1.41E-02	-1	-12
LOC220077	216386_at	66.11	-0.05	0.00	1.41E-02	-69	-6
HIPK2	224066_s_at	64.56	-0.01	-0.04	1.41E-02	-5	-17
USE1	219348_at	63.66	0.00	-0.02	1.41E-02	-2	-14
SERP2	239890_s_at	56.51	0.06	0.02	2.24E-02	97	20
TELO2	209528_s_at	54.35	0.09	0.03	2.24E-02	105	27
GTDC1	238585_at	54.07	0.04	-0.07	2.24E-02	21	-27
ZBTB14	208199_s_at	53.87	0.07	-0.07	2.24E-02	150	-60
ADRA2C	206128_at	53.27	0.11	0.01	2.24E-02	42	6
C16orf54	1559584_a_at	53.22	0.04	-0.01	2.24E-02	20	-30
KMT2E	223190_s_at	50.62	0.04	-0.01	2.72E-02	22	-13
VAPB	225923_at	49.28	-0.02	0.00	2.94E-02	-14	-6

Table 5 (continued)

Gene symbol	Probe set	<i>F</i> value	Mean change		BH <i>p</i> value	% fold change	
			female	male		female	male
MTG2	226854_at	48.54	0.01	−0.02	3.00E−02	10	−16
HIPK1-AS1	1570082_x_at	47.65	0.11	−0.03	3.11E−02	315	−17
SGCB	205120_s_at	47.25	0.03	0.00	3.11E−02	84	−2
LINC03048	234845_at	46.69	0.05	−0.01	3.16E−02	39	−10
SDCCAG8	1553034_at	46.18	0.04	−0.02	3.19E−02	26	−8
CALD1	201616_s_at	45.18	−0.06	−0.12	3.30E−02	−30	−53
ATN1	1555754_s_at	44.55	0.09	0.01	3.30E−02	66	8
DENND1A	219763_at	44.28	−0.03	0.05	3.30E−02	−26	162
RARA	1565358_at	44.25	0.05	−0.09	3.30E−02	22	−35
SORBS3	207788_s_at	43.97	0.05	−0.04	3.30E−02	38	−25
CAMSAP1	220410_s_at	43.77	−0.05	0.02	3.30E−02	−42	28
GPR157	227970_at	43.31	0.00	−0.03	3.30E−02	1	−27
SNHG29	244807_at	42.98	0.05	0.10	3.30E−02	27	183
RIOX2	1554774_at	42.88	0.06	−0.05	3.30E−02	33	−36
HSD17B12	1559518_at	42.65	0.02	0.02	3.30E−02	19	30
BCL2L11	1553088_a_at	42.34	0.04	−0.03	3.32E−02	59	−22
NRG2	206879_s_at	41.59	0.07	−0.02	3.51E−02	29	−11
MGARP	223734_at	40.69	−0.04	0.00	3.72E−02	−36	4
OSBPL6	238575_at	40.48	0.03	0.00	3.72E−02	138	−2
SMCO1	1559429_a_at	40.36	0.07	−0.03	3.72E−02	90	−26
TRMO	222195_s_at	40.04	0.02	0.03	3.72E−02	23	55
EDRF1	208115_x_at	39.99	0.00	0.02	3.72E−02	3	13
NEURL1	204889_s_at	39.36	0.13	−0.02	3.89E−02	126	−18
ZNF224	232427_at	39.16	−0.06	0.01	3.89E−02	−50	40
FASLG	211333_s_at	39.02	0.02	−0.06	3.89E−02	12	−19
MYH3	205940_at	38.02	0.02	−0.05	4.29E−02	17	−34
DHODH	217647_at	37.60	0.06	0.12	4.42E−02	68	161
GAB1	225998_at	36.41	−0.03	−0.07	4.79E−02	−19	−30
SMIM14-DT	1561432_at	36.12	0.04	0.00	4.79E−02	96	−4
PLB1	1553310_at	36.07	−0.14	0.02	4.79E−02	−68	19
CEP128	1557755_at	36.02	0.06	−0.02	4.79E−02	42	−15
OR5E1P	1566276_at	35.94	−0.01	−0.02	4.79E−02	−6	−23
MAPK1IP1L	212497_at	35.78	0.01	−0.08	4.79E−02	14	−57
WDR91	218971_s_at	35.70	−0.02	0.01	4.79E−02	−36	16
B4GALT1	201882_x_at	35.69	0.01	0.01	4.79E−02	14	8
SNIP1	235837_at	35.32	0.08	−0.05	4.94E−02	76	−35

SD, sleep deprivation; BH, Benjamini-Hochberg false discovery rate adjustment.

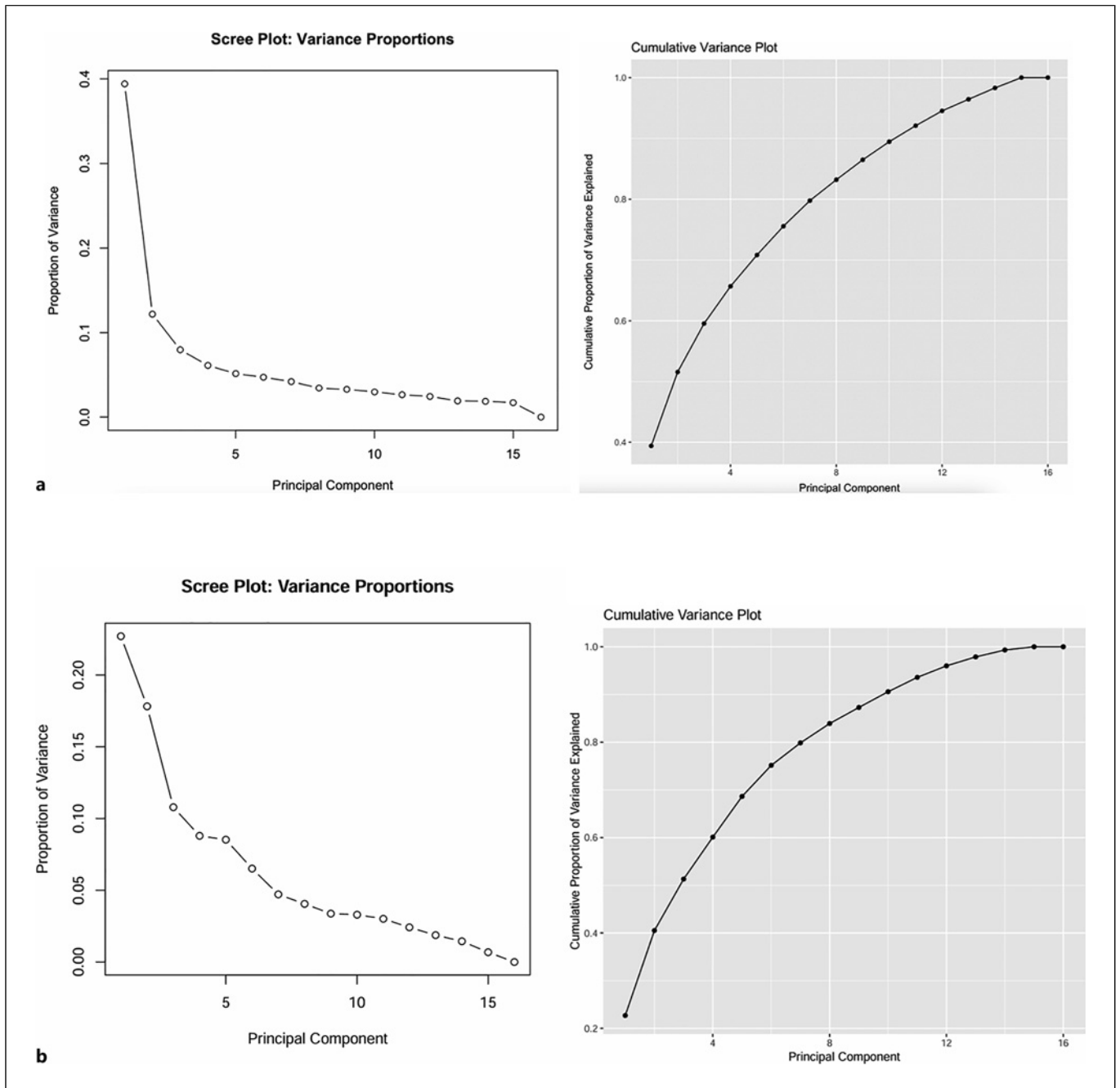


Fig. 1. a Gene expression PCA plots. **b** Brain region PCA plots.

The additional gene expression principal components (PC3_gene, PC4_gene, PC6_gene, PC7_gene, and PC8_gene) had functional clusters that emphasize the broad and multifaceted impact on the immune system. PC3_gene is enriched in viral defense mechanisms, apoptosis, and immune responses, while PC4_gene shows enrichment in general and adaptive

immunity, mitosis, and antigen binding. PC6 has significant functional clusters for immunoglobulin-like responses and B-cell receptor pathways; PC7_gene has significant functional clusters for antiviral defense and protective skin barriers, and PC8_gene for neutrophil activity and secretory processes, all relating to immune function.

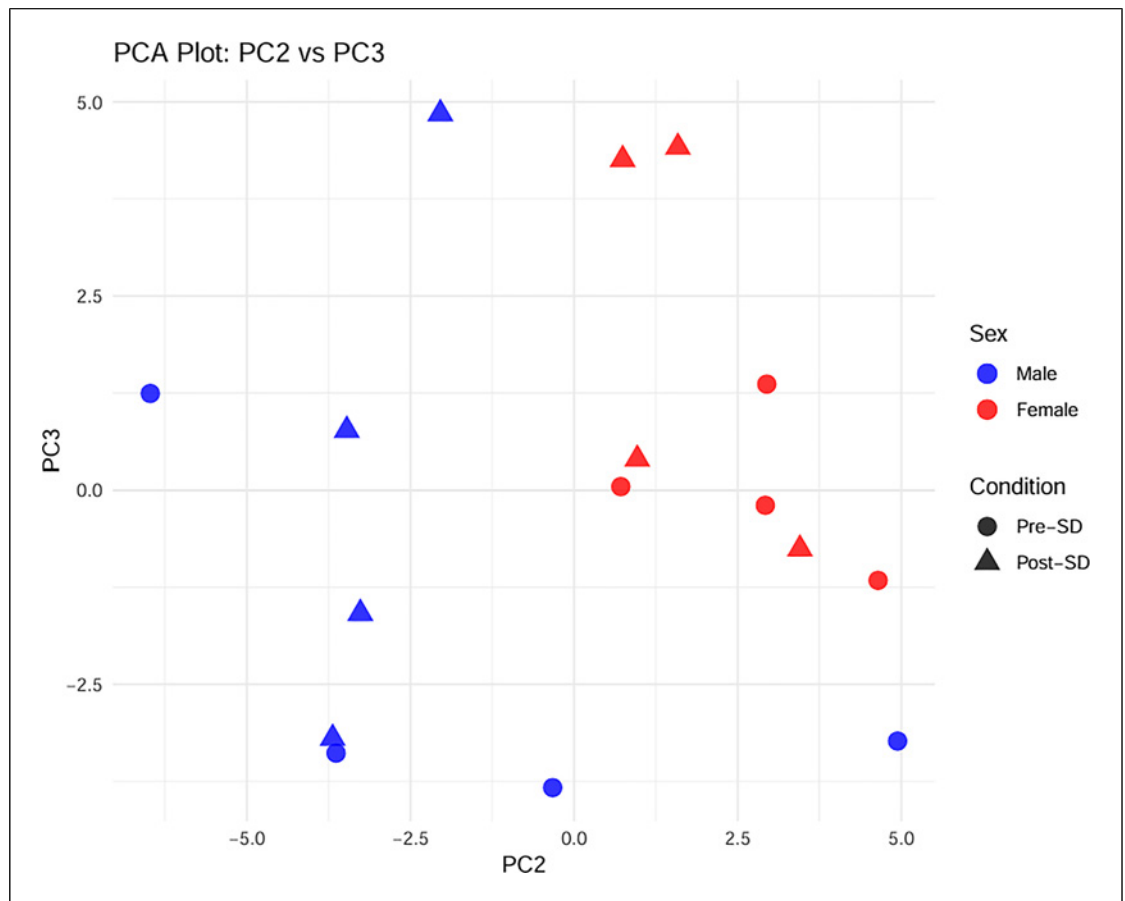


Fig. 2. PC2 versus PC3 scatter plot for ROI showing moderate grouping by sex.

Two-Way Mixed ANOVA for FDG-PET ROI

Our investigation into the effects of SD on regional brain metabolism utilized a two-way mixed ANOVA, considering sex as a between-subjects factor and condition (pre-SD vs. post-SD) as a within-subjects factor, and their interaction. The significant results from two-way mixed ANOVA with ROI-adjusted means are shown in Table 6. Two-way mixed ANOVA complete results are shown in online supplementary Table 6. ROI segmentation is shown in Figures 3–5.

Sex

There were 9 ROIs that showed nominal sex metabolic differences before multiple testing corrections (Table 6); however, none of these ROIs remained significant after the BH FDR correction.

Sleep Deprivation

Significant changes in metabolic activity (FDR $p < 0.05$) were observed across 11 brain regions, as shown in Table 6. Metabolism decreased with SD in the left insula,

left medial prefrontal cortex (BA32), left somatosensory cortex (BA1 and BA2), and left primary motor premotor cortex (BA6). Brain metabolism increased following SD in the right inferior longitudinal fasciculus, right primary visual cortex (BA17), right amygdala, right pons, left pons, right cerebellum, and left cerebellum (Fig. 3–5).

Interaction of Sex and SD

There were no ROIs with significant interaction effects between sex and SD for brain metabolism after BH FDR correction.

Two-Way Mixed ANOVA for Attention and Sleepiness Measures

The results of the two-way mixed ANOVA for the PVT showed a significant effect of sex ($F(1, 12) = 7.038, p = 0.021$) (Table 8). In contrast, the effects of SD ($F(1, 12) = 1.287, p = 0.279$) and the interaction between sex and SD ($F(1, 12) = 0.281, p = 0.606$) was not significant for the PVT. It is worth noting that previous research has shown that exercise

Brain region	F value		p value		BH		Mean										
	sex	cond	sex	cond	sex	cond	female	male	pre-SD	post-SD	female	male	pre-SD	post-SD			
Left somatosensory BA1 BA2	1.48	29.27	0.99	0.25	1.57E-04	0.34	0.69	0.01*	0.86	1.19	1.26	1.29	1.16	1.26	1.11	1.31	1.2
Right pons	4.23	25.46	0.41	0.06	2.86E-04	0.53	0.3	0.01*	0.86	0.86	0.81	0.77	0.9	0.8	0.92	0.74	0.88
Right cerebellum	0.09	15.5	0.5	0.77	1.98E-03	0.49	0.93	0.04*	0.86	0.95	0.93	0.89	0.99	0.91	0.99	0.88	0.99
Left pons	3.91	13.96	0.25	0.07	2.84E-03	0.63	0.3	0.04*	0.93	0.85	0.79	0.76	0.88	0.8	0.9	0.72	0.85
Left insula	0.26	12.88	0.55	0.62	3.72E-03	0.47	0.93	0.04*	0.86	1.42	1.38	1.45	1.35	1.46	1.38	1.45	1.32
Left primary motor premotor or BA6	0.57	12.21	0.16	0.46	4.43E-03	0.7	0.83	0.04	0.95	1.45	1.52	1.57	1.4	1.52	1.38	1.61	1.43
Right occipital cortex primary visual BA17	4.03	11.92	0.51	0.07	4.78E-03	0.49	0.3	0.04*	0.86	1.27	1.46	1.29	1.44	1.18	1.35	1.4	1.52
Right amygdala	0.08	11.9	2.49	0.78	4.81E-03	0.14	0.93	0.04*	0.86	0.73	0.72	0.66	0.8	0.7	0.77	0.62	0.82
Left cerebellum	0.08	10.38	0.02	0.78	0.01	0.89	0.93	0.05*	0.95	0.89	0.88	0.85	0.92	0.86	0.92	0.84	0.91
Right inferior longitudinal fasciculus	4.37	10.87	0.5	0.06	0.01	0.49	0.3	0.04*	0.86	0.69	0.6	0.57	0.72	0.63	0.75	0.51	0.69
Left medial prefrontal superior BA32	1.2	10.98	1.24	0.29	0.01	0.29	0.69	0.04*	0.86	1.49	1.43	1.52	1.4	1.53	1.45	1.51	1.36
BA, Brodmann area; cond, condition; SD, sleep deprivation; sex cond, sex-condition interaction. *FDR $p < 0.05$.																	

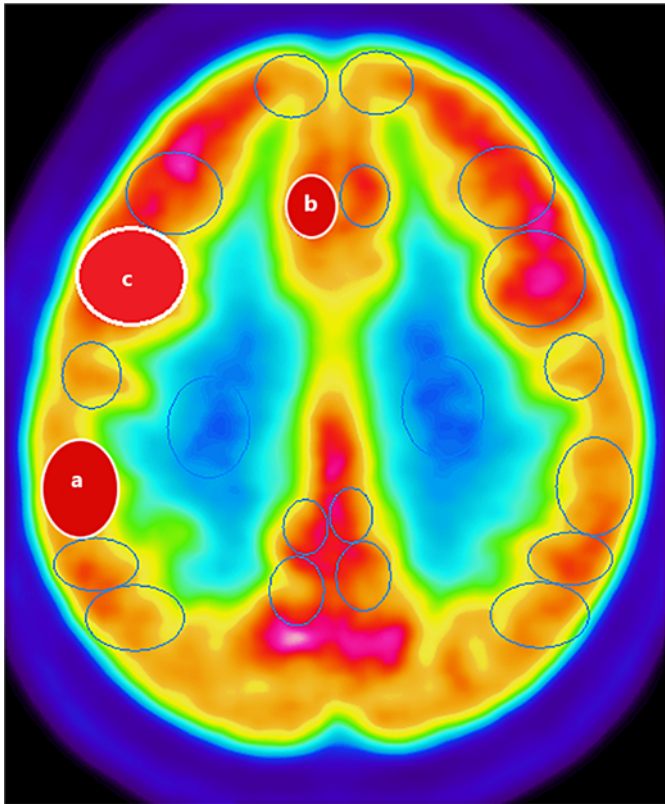


Fig. 3. Labeling of segmentation ROI (region of interest) with significant SD effect on plane 28. **a** Left somatosensory BA1/BA2. **b** Left medial prefrontal superior BA32. **c** Left primary motor premotor BA6/BA4. Red fill indicates increased metabolism following SD.

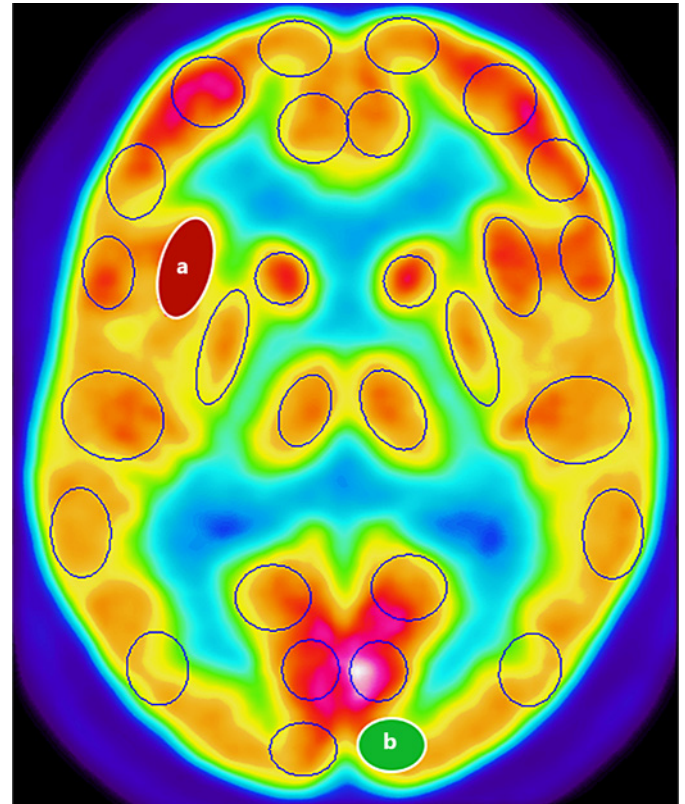


Fig. 4. Labeling of segmentation ROI (region of interest) with significant SD effect on plane 40. **a** Left insula. **b** Right occipital cortex primary visual BA17. Red fill indicates increased metabolism following SD. Green fill indicates decreased metabolism following SD. BA, Brodmann area.

training can significantly reduce errors and increase the speed on the PVT during total SD and recovery [35]. These findings raise the possibility that differences in exercise training between sexes could act as a confounding factor in PVT performance. However, exercise level and training types were not evaluated in this study. The potential influence of exercise should be considered due to established differences in fitness habits between sexes, which may have contributed to the observed variations in PVT performance. Future studies incorporating individual exercise history and training regimens may help further elucidate this effect. For the SSS, the effect of sex was not significant ($F(1, 6) = 0.070$, $p = 0.796$), but SD had a highly significant effect ($F(1, 6) = 33.800$, $p = 8.29E-05$) (Table 8). The interaction between sex and SD for the SSS was also not significant ($F(1, 6) = 0.200$, $p = 0.663$). These results suggest that while sex variable significantly impacts PVT performance, SD significantly affects SSS scores. There is no significant interaction between sex and SD for either measure.

PCA Loadings for ROI

The first 8 principal components captured 84% of the variance in FDG-PET data across brain ROI, as illustrated in Figure 5. For gene expression PCA, the first 4 principal components accounted for 66% of the variance, while for brain region ROI, the first 4 principal components account for 60% of the variance. ROI loadings for the first 8 PC_ROIs are shown in online supplementary Table 7. The top loading regions for PC1, the right occipital cortex primary visual (BA17), right cerebellum, left cerebellum, and left medial prefrontal superior (BA32) also show significant SD effects. Metabolism in the right occipital cortex primary visual (BA17), right cerebellum, and left cerebellum increased with SD and had positive loading values for PC1_ROI. Meanwhile, metabolism in the left medial prefrontal superior (BA32) decreased with SD, and there was a negative loading value in PC1_ROI. There is an overlap between significant ANOVA findings and top loadings for principal components 1 and 2 for ROI (Table 9).

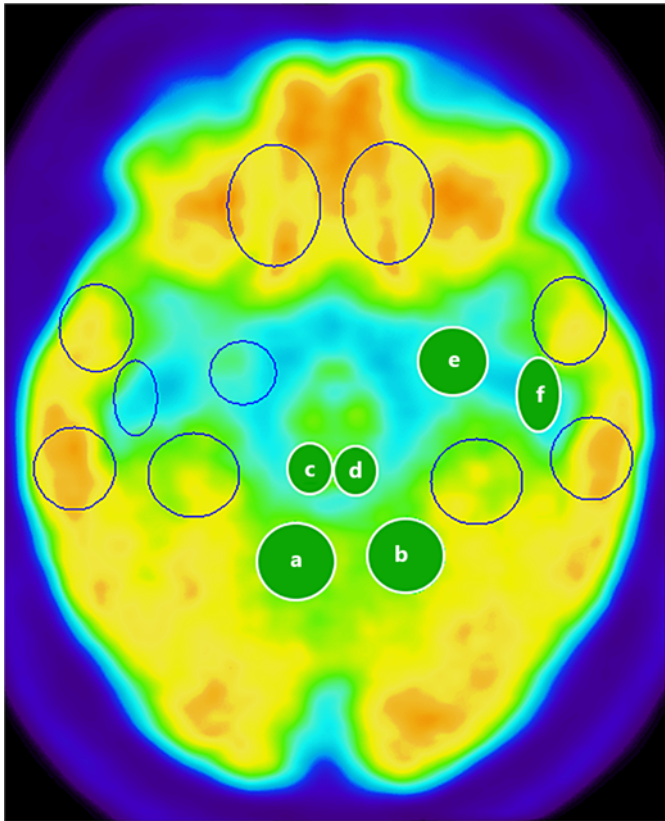


Fig. 5. Labeling of segmentation ROI (region of interest) with significant SD effect on plane 55. **a** Left cerebellum. **b** Right cerebellum. **c** Left pons. **d** Right pons. **e** Right amygdala. **f** Right inferior longitudinal. Green fill indicates increased metabolism following SD.

PCA of regional brain metabolism also revealed a separation of male and female participants along PC2 and PC4, suggesting sex-related differences in brain metabolic responses. A scatter plot of PC2 versus PC4 for ROI (Fig. 6) visually illustrates this clustering by sex, highlighting the importance of evaluating sex as a factor in SD effects on brain metabolism.

Principal Components for Brain Metabolism and Peripheral Gene Expression Are Correlated

The relationship between the first 8 principal components from gene expression and FDG-PET ROI, along with PVT, SSS, sex, age, and SD, were examined by post hoc correlations (Table 7). A significant positive correlation was found between SD (pre-SD coded as 1, post-SD coded as 2) and SSS ($r(16) = 0.77, p < 0.001$), also confirmed by two-way mixed ANOVA (Table 8). Additionally, a significant positive correlation was observed between sex (coded as 1 for males and 2 for females) and PVT per-

formance ($r(16) = 0.58, p = 0.02$), indicating that females have higher PVT scores (longer response time) than males and confirmed by two-way mixed ANOVA (Table 8). Significant positive correlations were found between PC1_ROI loadings and both SD ($r(16) = 0.56, p = 0.02$) and age ($r(16) = 0.58, p = 0.02$). Males had higher overall PC1_ROI values than females, shown in the negative correlation of PC1_ROI and sex ($r(16) = -0.55, p = 0.03$). Females showed higher metabolism in brain areas associated with PC2_ROI ($r(16) = 0.56, p = 0.02$).

Between the major principal components and measures of attention, a significant positive correlation was also observed between performance on PVT and PC4_gene ($r(16) = 0.53, p = 0.03$). Meanwhile, SSS was found to be correlated positively with PC8_gene ($r(16) = 0.58, p = 0.02$) and negatively correlated with PC6_ROI ($r(16) = -0.58, p = 0.02$). These findings suggest that SD, as reflected by increased SSS scores and PVT response times, may influence gene expression patterns, particularly those linked to PC4_gene and PC8_gene. The strongest significant correlation was observed to be between PC2_ROI and PC4_gene ($r(16) = 0.65, p = 0.01$). ROI and PCA gene expression correlation results are shown in online supplementary Table 8.

Regions contributing to the observed correlation between PC1_ROI and SD (Table 9) were the right occipital cortex, primary visual cortex BA17, and right and left cerebellum, which showed positive increased metabolic activity following SD. The left medial prefrontal superior BA32 showed a significant decrease in metabolic activity post-SD. Specifically, males exhibited higher loadings in regions such as the right occipital cortex (primary visual BA17) and the left thalamus, while females showed higher loadings in regions with significant negative effects, including the left medial prefrontal cortex superior BA32 and right frontal cortex BA10 (Table 9). Females showed higher metabolism in brain areas associated with PC2_ROI such as the left frontal cortex BA45, and the right orbital frontal BA11 regions, and with increased expression of genes associated with PC4_gene ($r(16) = 0.85, p = 3.00E-05$) (Table 7). Out of the top loadings for PC2_ROI, the left frontal cortex BA45, right orbital frontal BA11, left temporopolar BA38, and right temporopolar BA38 ROI also showed a nominally significant sex effect (Table 9).

To further explore the association between PC2_ROI and PC4_gene, functional enrichment of PC4_gene positive and negative features was tested for over-enrichment using DAVID. Genes with positive loadings for PC4_gene were enriched for functional clusters related to antiviral defense and innate immunity, while those with negative loadings were enriched for mitosis (cell cycle) and adaptive

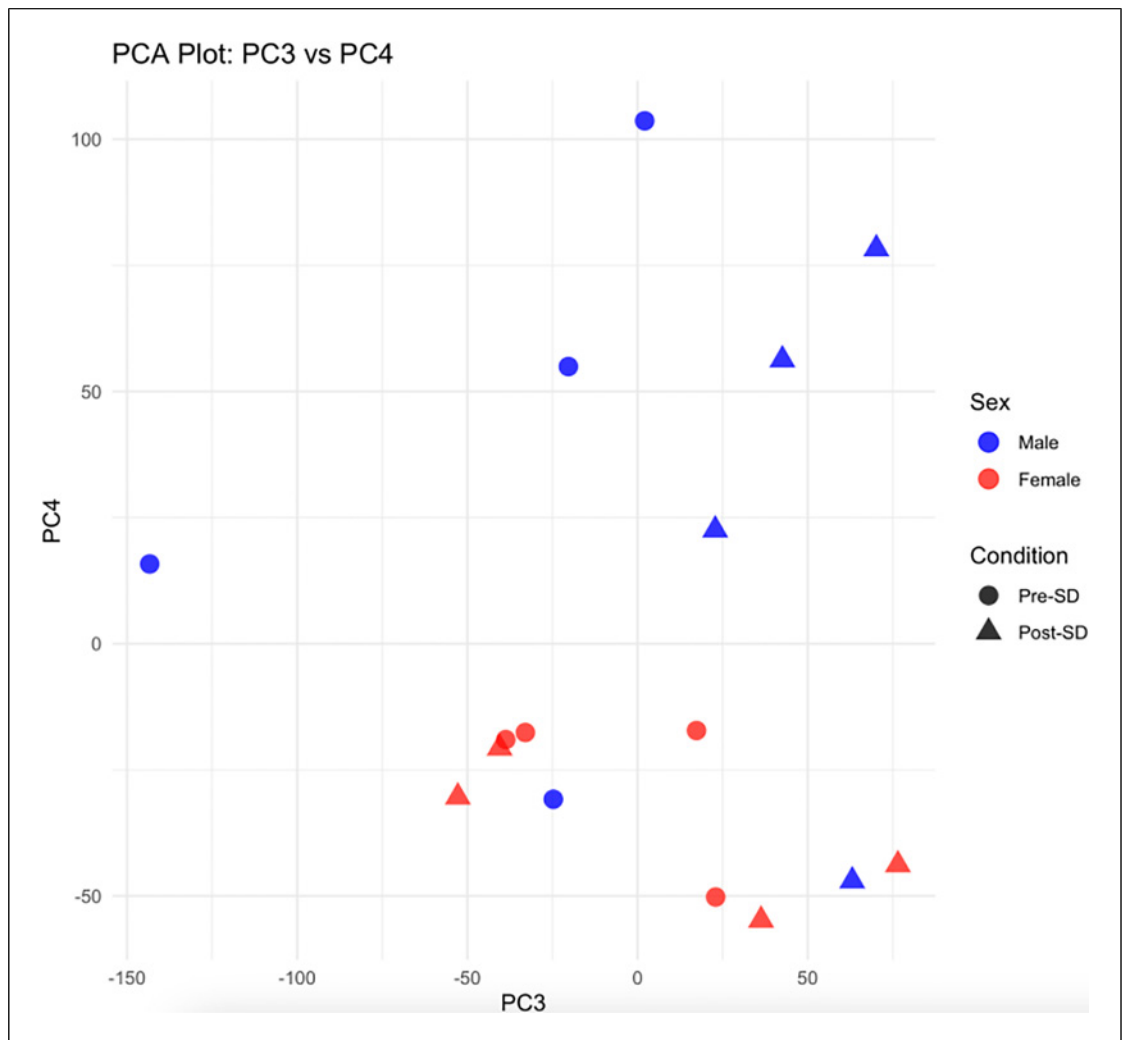


Fig. 6. PC2 versus PC3 scatter plot for gene expression suggesting moderate grouping by sex.

immunity. These results suggest that SD may differentially affect immune-related gene expression, potentially driven by heightened innate immune responses and suppressed adaptive immune activity. Given the positive correlation between SSS and PC8_gene, it is plausible that subjective sleepiness reflects downstream effects of disrupted immune regulation and metabolic stress on gene expression. However, a post hoc correlation matrix analysis of PC4_gene features with PC2_ROI features did not reveal a stronger association than the overall PC2_ROI-PC4_gene relationship, indicating that these effects may involve broader network interactions rather than direct gene-region pairings.

Additionally, an additional post hoc correlation matrix was constructed using combined metabolic values from all ROIs and the top 300 genes, showing the strongest SD effects

from the two-way mixed ANOVA (online suppl. Table 9). The right parahippocampus ROI nominally tracked with the most genes (112 probes out of 300, with 111 unique genes). The next highest region was the left primary motor premotor or BA6, with 102 probes and 94 unique genes, with 61 unique genes shared between the two regions. This suggests a relationship between metabolism in these brain regions and gene expression changes associated with SD. Moreover, we found nominally significant enrichment for cross-linking lysine-glycine isopeptide bonds and ubiquitin modification (interchain with G-Cter in SUMO2) functional clusters in the top SD genes correlated with right parahippocampus, while significant (BH p value <0.05) enrichment for early endosome and alcoholism functional clusters were found for genes for the left primary motor premotor BA6 associated with biological response to SD (online suppl. Table 9).

Table 7. Correlation and significance matrix (upper diagonal *r*/lower diagonal *p* value)

	Cond	Sex	Age	SSS	PVT	PC1 gene	PC2 gene	PC3 gene	PC4 gene	PC5 gene	PC6 gene	PC7 gene	PC8 gene	PC1 ROI	PC2 ROI	PC3 ROI	PC4 ROI	PC5 ROI	PC6 ROI	PC7 ROI	PC8 ROI
Condition	-	0.000	0.000	0.767	0.250	0.004	-0.407	0.441	0.022	0.356	0.300	0.333	0.345	0.563	0.254	-0.245	-0.445	0.226	-0.261	0.363	-0.134
Gender	1.000	-	-0.447	0.059	0.584	-0.237	0.196	0.076	0.852	0.306	-0.315	-0.103	0.011	-0.548	0.561	-0.406	0.035	0.153	-0.120	-0.059	0.115
Age	1.000	0.083	-	-0.324	-0.387	-0.275	0.152	0.186	-0.106	-0.625	0.563	0.345	-0.306	0.580	-0.134	-0.143	-0.039	-0.157	0.441	-0.177	-0.028
SSS	0.001	0.828	0.220	-	0.148	0.332	-0.183	0.256	-0.038	0.451	-0.007	0.308	0.581	0.436	0.285	-0.006	-0.214	0.091	-0.575	0.250	0.103
PVT	0.351	0.017	0.139	0.584	-	-0.263	-0.196	-0.034	0.534	0.313	-0.186	0.090	0.138	-0.405	0.080	-0.482	-0.427	0.323	-0.224	0.027	0.381
PC1_gene	0.988	0.376	0.303	0.209	0.326	-	-0.006	0.321	-0.498	0.226	-0.440	0.065	-0.014	0.110	-0.120	-0.036	0.300	-0.350	-0.145	0.025	-0.064
PC2_gene	0.118	0.467	0.574	0.497	0.467	0.983	-	0.000	0.000	0.000	0.000	0.000	0.000	-0.117	-0.169	0.019	0.532	-0.035	-0.102	-0.063	0.172
PC3_gene	0.087	0.779	0.491	0.338	0.901	0.225	1.000	-	0.000	0.000	0.000	0.000	0.000	0.368	0.204	-0.474	0.141	0.002	-0.156	-0.199	-0.405
PC4_gene	0.937	0.000	0.697	0.889	0.033	0.049	1.000	1.000	-	0.000	0.000	0.000	0.000	-0.418	0.647	-0.353	-0.248	0.087	0.031	-0.180	0.136
PC5_gene	0.176	0.249	0.010	0.079	0.237	0.399	1.000	1.000	1.000	-	0.000	0.000	0.000	-0.192	0.093	0.037	-0.105	-0.195	-0.417	0.473	0.078
PC6_gene	0.259	0.234	0.023	0.980	0.491	0.088	1.000	1.000	1.000	1.000	-	0.000	0.000	0.398	0.035	0.297	-0.479	-0.052	0.151	0.072	-0.003
PC7_gene	0.207	0.704	0.191	0.246	0.740	0.810	1.000	1.000	1.000	1.000	1.000	-	0.000	0.385	0.060	-0.409	0.008	-0.286	-0.067	0.495	0.313
PC8_gene	0.190	0.967	0.250	0.018	0.611	0.959	1.000	1.000	1.000	1.000	1.000	1.000	-	0.077	0.097	0.189	-0.089	0.579	-0.521	0.190	0.108
PC1_ROI	0.023	0.028	0.019	0.092	0.119	0.686	0.667	0.160	0.107	0.475	0.126	0.141	0.776	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PC2_ROI	0.342	0.024	0.620	0.285	0.768	0.658	0.530	0.448	0.007	0.733	0.897	0.826	0.722	1.000	-	0.000	0.000	0.000	0.000	0.000	0.000
PC3_ROI	0.361	0.118	0.598	0.981	0.059	0.895	0.944	0.063	0.180	0.892	0.263	0.115	0.484	1.000	1.000	-	0.000	0.000	0.000	0.000	0.000
PC4_ROI	0.084	0.897	0.885	0.425	0.099	0.259	0.034	0.603	0.355	0.698	0.060	0.976	0.742	1.000	1.000	1.000	-	0.000	0.000	0.000	0.000
PC5_ROI	0.400	0.570	0.563	0.737	0.222	0.184	0.898	0.993	0.749	0.469	0.848	0.282	0.019	1.000	1.000	1.000	1.000	-	0.000	0.000	0.000
PC6_ROI	0.329	0.658	0.087	0.020	0.403	0.592	0.706	0.564	0.910	0.108	0.577	0.806	0.039	1.000	1.000	1.000	1.000	1.000	-	0.000	0.000
PC7_ROI	0.168	0.827	0.512	0.350	0.920	0.928	0.817	0.461	0.504	0.064	0.792	0.051	0.480	1.000	1.000	1.000	1.000	1.000	1.000	-	0.000
PC8_ROI	0.621	0.671	0.917	0.703	0.145	0.814	0.525	0.120	0.615	0.775	0.991	0.238	0.692	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-
Pre-SD coded as 1, post-SD coded as 2. Sex: males coded as 1, females coded as 2. Cond, condition. Bolded values represent significant positive correlation R values. Italicized values represent significant negative correlation R values. Significance threshold <i>p</i> < 0.05.																					

Pre-SD coded as 1, post-SD coded as 2. Sex: males coded as 1, females coded as 2. Cond, condition. Bolded values represent significant positive correlation *R* values. Italicized values represent significant negative correlation *R* values. Significance threshold *p* < 0.05.

Table 8. Two-way mixed ANOVA on cognitive response measures

	num df	den df	F	p value
PVT				
Sex	1	12	7.038	0.021
SD	1	12	1.287	0.279
Sex × SD	1	12	0.281	0.606
SSS				
Sex	1	6	0.070	0.796
SD	1	6	33.800	8.293E−05
Sex × SD	1	6	0.200	0.663

PVT, Psychomotor Vigilance Task; SSS, Stanford Sleepiness Scale; num df, numerator degrees of freedom; den df, denominator degrees of freedom; F, F statistic; p value, statistical significance.

Interaction of Laterality and SD by Brain Region

Several brain regions showed a significant interaction between laterality and SD, although these interactions did not pass FDR. As an example, the lateral temporal region, BA21, demonstrated an interaction between hemisphere and SD ($F(1, 7) = 9.10, p = 0.02$, right > left post-SD vs. right < left before SD); the occipital cortex primary visual BA17 showed interaction ($F(1, 7) = 6.65, p = 0.04$, right > left post-SD vs. right < left before SD). Similarly, the inferior longitudinal fasciculus showed an interaction ($F(1, 7) = 6.69, p = 0.04$, right > left post-SD vs. right < left before SD); the cerebellum exhibited a laterality SD interaction ($F(1, 7) = 8.01, p = 0.03$, increased laterality right > left post-SD). These findings indicate that SD may differentially impact neural processing based on the hemisphere, particularly in occipital, temporal, and cerebellar functioning (online suppl. Table 10).

Discussion

A total of 11 unique genes showed a significant differential expression for sex, 27 genes showed a significant differential expression after SD, and 55 genes showed a significant sex and SD interaction effect. Among the top pathways, UBL conjugation was the most enriched gene cluster associated with SD, underscoring its widespread role in cellular processes.

UBL conjugation is a post-translational modification process where proteins are tagged with ubiquitin-like molecules, which regulates several key cellular functions [36]. These include chromatin dynamics and transcription regulation [37]. The downregulation of

several histone genes (H3C10, H2BC6, H2BC9, H2BC5, H2BC12) after SD suggests a potential reduction in chromatin remodeling and transcriptional activity. Such changes may affect chromatin accessibility for transcription factors and other regulatory proteins [38].

Similarly, the downregulation of SMCHD1 and WASHC1 in response to SD suggests significant alterations in chromatin structure and other nuclear processes [39, 40]. In addition, key genes involved in stress response and cellular homeostasis, such as SGK1 and FKBP5, show differential expression. While SGK1 is downregulated, FKBP5 is upregulated, indicating a complex modulation of stress-related pathways [41]. FKBP5 has consistently been implicated in both human [42] and animal studies [43] as a reliable biomarker for stress. Our findings align with existing research that underscores FKBP5’s role in stress-related processes, including apoptosis [44] and SD-induced stress responses [42].

The involvement of UBL conjugation in protein stability and turnover further emphasizes its role in cellular adaptation to SD. This process is energy intensive as it manages protein homeostasis by targeting misfolded or damaged proteins for degradation [45, 46] – a function crucial in neurons sensitive to protein misfolding and limited in repair capacity. The upregulation of OTULIN, a deubiquitinase, may reflect a protective mechanism against stress-induced protein degradation. At the same time, the downregulation of ITCH, an E3 ubiquitin ligase, suggests shifts in protein degradation pathways [47, 48]. Together, these findings indicate that the ubiquitin-proteasome system adapts to SD by adjusting protein stability and degradation mechanisms, though the high metabolic demands of this response may deprioritize other cellular processes.

In addition to its roles in transcription and protein turnover, UBL conjugation intersects with immune pathways. For instance, the upregulation of CISH, a cytokine signaling regulator, reflects an altered immune response under SD. At the same time, the downregulation of immune response genes like GBP1 and CYBB points to a nuanced shift, potentially prioritizing energy conservation over immune readiness [49–51]. PCA of gene expression patterns in PC4_gene showed enrichment of immunity-related clusters, with prominent differences in immune and antiviral defense genes by sex. Females, who often display stronger immune responses and higher risks of autoimmune diseases, may have immune responses particularly affected by these changes [52].

Additionally, PC4_gene was enriched for mitosis-related genes, including NDC80 [53, 54], NEK2 [55, 56], and CDK1 [57, 58], suggesting sex-specific

Table 9. Analysis of SD and sex effects on PC1 PC2 brain region loading

PC1	SD effect				
positive	loading coeff	% change	negative	loading coeff	% change
Right occipital cortex primary visual BA17	0.231	11.6	Left medial prefrontal superior BA32	-0.155	-7.9
Left occipital cortex 2nd visual area BA19	0.206	6.1	Right caudate	-0.161	-5.4
Right occipital cortex 2nd visual area BA19	0.188	1.7	Right insula	-0.172	-12.8
Right temporal cortex fusiform BA37	0.186	6.9	Left frontal cortex BA46	-0.176	-12.6
Right cerebellum	0.162	11.2	Right medial prefrontal superior BA32	-0.182	27.3
Left thalamus	0.162	4.4	Left medial prefrontal cortex BA32	-0.198	-6.1
Right posterior cingulate	0.161	7.0	Right frontal cortex BA10	-0.211	-9.3
Left cerebellum	0.157	8.2	Left frontal cortex BA10	-0.225	-5.5
PC1	Sex effect				
positive	loading coeff	% difference (male vs. female)	negative	loading coeff	% difference (male vs. female)
Right occipital cortex primary visual BA17	0.231	15.0	Left medial prefrontal superior BA32	-0.155	-4.0
Left occipital cortex 2nd visual area BA19	0.206	11.2	Right caudate	-0.161	0.0
Right occipital cortex 2nd visual area BA19	0.188	16.0	Right insula	-0.172	3.5
Right temporal cortex fusiform BA37	0.186	8.7	Left frontal cortex BA46	-0.176	-2.6
Right cerebellum	0.162	-2.1	Right medial prefrontal superior BA32	-0.182	-12.5
Left thalamus	0.162	5.1	Left medial prefrontal cortex BA32	-0.198	-7.6
Right posterior cingulate	0.161	10.8	Right frontal cortex BA10	-0.211	-7.8
Left cerebellum	0.157	-1.1	Left frontal cortex BA10	-0.225	-10.0
PC2	Sex effect				
positive	loading coeff	% difference (male vs. female)	negative	loading coeff	% difference (male vs. female)
Left frontal cortex BA45	0.204	-5	Left frontal pole BA9	-0.159	-3.1
Right orbital frontal BA11	0.195	-7.0	Right parietal cortex supramarginal gyrus BA40	-0.174	11.5
Left angular gyrus parietal B39	0.189	-0.7	Left putamen	-0.176	0.7

Table 9 (continued)

PC2	Sex effect				
	loading coeff	% difference (male vs. female)	negative	loading coeff	% difference (male vs. female)
positive					
Left temporopolar BA38	0.186	−15.5	Right somatosensory BA1 BA2	−0.199	4.8
Right thalamus	0.179	1.5	Left corona radiata	−0.206	−14.6
Right temporopolar BA38	0.171	−15.3	Right corona radiata	−0.216	−5.3
Right lateral temporal BA21	0.164	−2.6	Right DLPC BA46	−0.235	7.9
Left thalamus	0.155	5.1	Left DLPC BA46	−0.245	9.2

Areas with significant SD effect from 2-way mixed ANOVA are bolded. Areas with significant sex effect from 2-way mixed ANOVA are bolded.

regulatory influences on cell division, as PC4_gene was strongly correlated with sex. Although these genes were downregulated following SD, the lack of a direct correlation with sleep conditions implies that their expression remains generally stable across sleep states. However, sex-based differences in their expression may offer insights into sex-specific risks for diseases involving cell proliferation, such as cancer. To further investigate the effects of SD, we divided PC4_gene into subsets of genes that were upregulated or downregulated following SD. We observed that the upregulated subset was enriched with genes involved in antiviral and innate immunity functions, including HERC5 [59], DDX60 [60], MX1 [59], IFI6, IFI44L, IFIT1, IFIT2, IFIT3, and IFIT5 [61]. This pattern suggests an enhanced antiviral state as a compensatory mechanism to counteract the physiological stress induced by SD. Conversely, the downregulated subset showed enrichment for mitosis-related and adaptive immunity genes, such as IGHD, IGHG1, IGHM, and T-cell receptors (e.g., TRDC, TRDV3), as well as SLAMF7 and TNFRSF17, which are involved in lymphocyte activation and B-cell maturation. This shift in gene expression suggests a potential prioritization of innate immune responses over adaptive immunity, possibly as an energy-saving response to SD.

These findings align with research on the relationships between sleep, immunity, and sex differences. For example, SD has been found to elevate inflammatory markers, and females in general appear to display more robust immune responses, including higher IL-6 and TNF- α levels [62]. Females' higher susceptibility to autoimmune diseases and female SD effects [63, 64] indicate that immune gene expression changes should be ad-

dressed with sex-targeted interventions. Additionally, PC1_ROI and PC2_ROI were significantly correlated with sex, underscoring the importance of considering sex-specific effects in these regions when evaluating immune and neurometabolic responses to SD.

To explore the functional impact of SD in the brain, we examined the left primary motor premotor area (BA6), which exhibited a significant decrease in metabolism following sleep deprivation (FDR $p < 0.05$) and also had nominally significant correlations with 94 unique genes from the top 300 condition effect genes. Prior studies indicate that BA6 exhibits heightened slow-wave activity following learning tasks, reflecting its role in memory consolidation and recovery processes likely disrupted by SD [65]. Additionally, while left BA6 is generally more active in resting states than during tasks, this difference disappears in sleep-deprived individuals [66], suggesting that SD disrupts the default mode network, hindering BA6's engagement in recovery and integration processes critical for cognitive and motor functions. This disruption could reduce the region's efficiency during rest, linking sleep loss to impaired metabolic and functional dynamics in BA6. Furthermore, neurons in the BA6 may be more vulnerable to stress-induced metabolic decline due to their reliance on oxidative metabolism and limited defenses against protein misfolding and oxidative damage [67]. The altered peripheral expression of UBL conjugation genes in response to SD may reflect disrupted protein regulation pathways critical for maintaining cellular health, affecting sensitive brain regions such as BA6.

Moreover, enrichment for early endosome and alcoholism functional clusters was found among the gene expressions that correlated with BA6 metabolic activity.

This included genes such as TFRC, ITCH, WASF2, WASHC1, CNTNAP2, RUSC1, GGA1, F2RL1, and VPS26A.

The upregulation of genes such as TFRC, which supports iron uptake for mitochondrial energy production [68], and ITCH, which facilitates protein degradation under stress [47, 48], reflects the primary motor cortex's heightened metabolic [69] and proteostatic [70] cellular stress during prolonged wakefulness [71]. These molecular adaptations likely mitigate the detrimental effects of SD while sustaining the energy-intensive processes required for motor control.

The correlation between SD and increased expression of genes such as WASF2, WASHC1, and CNTNAP2 highlights the role of synaptic plasticity and cytoskeletal dynamics in the primary motor cortex's response to stress [72, 73]. Synaptic remodeling and vesicle trafficking, regulated by genes like RUSC1, GGA1, and VPS26A [74–76], are energy-intensive processes essential for maintaining neurotransmission and motor learning under conditions of disrupted homeostasis. Additionally, genes involved in neuroprotection, such as F2RL1, mitigate the effects of oxidative stress and inflammation [77], further supporting the primary motor cortex's resilience during SD. These findings suggest that the left primary motor cortex relies on a coordinated molecular response to SD, with genes regulating energy metabolism, synaptic plasticity, and neuroprotection playing critical roles in its metabolic activity.

Overall, this study highlights the multifaceted impact of SD, where decreased metabolism, structural vulnerability, immune shifts, and protein regulation via ubiquitin-like modifications converge to disrupt regional brain function. The limited number of subjects may restrict the generalizability of the results and increase the risk of model overfitting, particularly in correlation analyses. Future studies with larger cohorts and independent replication are needed to validate these findings. Despite the preliminary nature of this research, these findings underscore the importance of further studies to replicate and expand upon these results. A deeper understanding of the mechanisms underlying stress and resilience in response to SD could inform strategies to mitigate its detrimental effects on the brain and overall health.

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Statement of Ethics

This study protocol was reviewed and approved by the Institutional Review Board of University of California, Irvine, Approval No. 2001-1616. Written informed consent was obtained from all participants before the start of this study.

Conflict of Interest Statement

Marquis P. Wawter was a member of the journal's Editorial Board at the time of submission.

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Author Contributions

LB processed and analyzed ROI metabolic data and performed statistical analyses. R.S. processed gene data and assisted in interpreting gene expression analysis. F.L. assisted in data collection, literature review, and preparation of figures and tables. M.P.V. and J.C.-S.W. contributed to the project's design and execution, guided data collection and analysis, and provided critical leadership and insights throughout the research. All authors participated in drafting and editing the manuscript.

Data Availability Statement

The supplementary tables, data that support the findings of this study, as well as the complete R code used for analysis can be found at <http://datadryad.org/stash/share/5cDCYPQd57-UuafYisnqdMuy3TCOeYVY-8W9x8BpSo>.

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