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Biomarkers for Noise-Induced Sleep Disruption

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12. Abstract Noise experienced by the general population in proximity to airports and aviation flight routes can result in disrupted sleep. Sleep disruption and fragmentation may be mitigated by wearing earplugs or introducing broadband noise (e.g., pink noise) into the bedroom. However, these countermeasures are poorly investigated and understood. The FAA ASCENT has supported the University of Pennsylvania in investigating earplugs and pink noise to mitigate sleep disruption from simulated aircraft noise, using approaches such as physiological and neurobehavioral performance monitoring. The FAA Civil Aerospace Medical Institute complemented these efforts by receipt of blood samples collected from human subjects exposed to the simulated aircraft noise and monitored by the University of Pennsylvania. Ribonucleic acid (RNA) was extracted from the blood followed by total RNA sequencing and differential gene expression analyses, which provided molecular insights about human responses to noise and the mitigations tested. Altogether 1,246 genes were differentially expressed in response to the experimental exposure condition (control without noise, pink noise at a level of 50 dBA, simulated aircraft noise, and simulated aircraft noise with a mitigation: pink noise at 40 dBA, pink noise at 50 dBA, or earplugs). There were 2,181 genes associated with awakenings during noise exposure.		
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List of Abbreviations

Acronym	Abbreviation Explained
General	
CAMI	Civil Aerospace Medical Institute
dBA	A-weighted decibels
FAA	Federal Aviation Administration
FDR	False Discovery Rate
RNA	Ribonucleic acid
Exposure Conditions	
AN	Simulated aviation noise exposure
AN+BN40	Simulated aviation noise exposure with pink noise at 40 decibels
AN+BN50	Simulated aviation noise exposure with pink noise at 50 decibels
AN+EP	Simulated aviation noise exposure with earplugs
CTRL	Control exposure (no noise)
Physiology and Performance Metrics	
Accuracy	Accuracy across Cognition battery
BP_Av_Dia	Average of 3 diastolic blood pressure measurements
BP_Av_Sys	Average of 3 systolic blood pressure measurements
DS_DA_AvRT	Divided Attention Task Average response time (seconds)
DS_SDLanePos	Lane position deviation (feet)
Hfpow_FFT_ms_sq	Heart Rate Variability (HRV) frequency-domain analysis: Absolute High-Frequency (HF) power derived from Fast Fourier Transform (FFT).
HRV_SDNN_ms	Heart Rate Variability (HRV) time-domain analysis: Standard Deviation (SD) of RR (SDNN) intervals in 5-minute segments.
MS_KSS	Score on Karolinska Sleepiness Scale from morning survey
MS_POMS_Total	Total score for entire Profile of Mood States from morning survey

PVT_Lapses	Number of lapses during Psychomotor Vigilance task (3 min PVT-B)
Speed	Speed across Cognition battery
SU_PoorSleepQual	Survey response to question of poor sleep quality
Polysomnography Metrics	
PSG_SOL	Sleep Onset Latency [min]
PSG_REML	Rapid Eye Movement sleep latency [min]
PSG_Wake	Time spent Awake [min]
PSG_N1	Time spent in sleep stage N1 [min]
PSG_N2	Time spent in sleep stage N2 [min]
PSG_N3	Time spent in sleep stage N3 [min]
PSG_REM	Time spent in REM sleep [min]
PSG_SE	Sleep efficiency [%]
PSG_nAWRperhTST	Number of awakenings per hour sleep
PSG_nAROperhTST	Number of EEG arousals per hour sleep
ORP_Av_All	Average Odds Ratio Product value across Time in Bed [measure of wake propensity ranging from 0-2.5]
ORP_Av_All_Noise	Average Odds Ratio Product value during noise periods only [measure of wake propensity ranging from 0-2.5]
PSG_nAWRperhTST_Noise	Number of awakenings per hour sleep [noise periods only]
PSG_nAROperhTST_Noise	Number of electroencephalogram arousals per hour sleep [noise periods only]
PSG_N3REM	Time spent in sleep stage N3 or Rapid Eye Movement sleep [min]

1. Introduction

Noise experienced by the general population in proximity to airports and aviation flight routes may result in disrupted sleep. Sleep disruption and fragmentation may be mitigated by wearing earplugs or introducing broadband noise (e.g., pink noise). However, these countermeasures are poorly investigated and understood. The Federal Aviation Administration (FAA) Center of Excellence for Alternative Jet Fuels and Environment (also known as the Aviation Sustainability Center or ASCENT) has supported the University of Pennsylvania in investigating earplugs and pink noise at levels of 40 A-weighted decibels (dBA) and 50 dBA to mitigate sleep disruption from simulated aircraft noise. These values are in the range of noise detected in people's bedrooms based on prior field research, with a median of approximately 45 dBA (Basner et al., 2023). Assessments encompassed physiological, neurobehavioral performance, and polysomnographic monitoring. The FAA Aviation Safety, Civil Aerospace Medical Institute (CAMI) complemented these efforts with gene expression analyses, providing additional information about human responses to simulated aircraft noise and mitigation approaches. In particular, CAMI tested for gene expression ribonucleic acid (RNA) biomarkers (biological indicators) of responses to experimental conditions. Gene expression changes were analyzed in association with simulated aircraft noise exposure in the presence or absence of mitigation approaches, in relation to physiological and performance changes following exposure, and in relation to scored polysomnography sleep assessments. Findings may be used to inform the analysis and interpretation of results from ongoing assessments of the effects of aircraft noise on the general public, including the FAA's National Sleep Study (Basner et al., 2023).

2. Methods

2.1. Study Design

Volunteer subjects were recruited to a multi-night study at the University of Pennsylvania, with Institutional Review Board approval by the University and by the FAA CAMI. A total of 26 subjects aged 21 to 41 years participated, with successful blood draws. Each subject was exposed to an initial noise-free adaptation night for study acclimation, followed by six separate exposure nights in randomized order. Exposures were designed to reflect simulated aircraft noise, with and without earplugs or pink noise as mitigations. Simulated aircraft noise was played back with maximum sound pressure levels ($L_{AS,max}$) of 45, 55, or 65 dBA, and consisted of a series of 93 events: 2 jets (from 2004-2006 DLR AIRORA study), (Basner et al., 2011); 2 helicopters (level and angled blades); 2 drones (fast and slow overflights); 1 low sonic boom (evanescent wave); 1 car (from 2004-2006 DLR AIRORA study); 1 train (from 2004-2006 DLR AIRORA study); 2 alarm sounds (fire alarm, baby crying). Each traffic noise event was played back three times at each noise level, and the fire alarm and baby crying sounds were played back twice at each noise level. Specifically, the six exposure nights involved exposure to simulated aircraft noise alone (AN), aircraft noise with earplugs (AN+EP), aircraft noise with pink noise at 40 dBA (AN+BN40), aircraft noise with pink noise at 50 dBA (AN+BN50), pink noise alone of 50 dBA (BN50), or a control with no introduced noise or earplugs. Subjects remained in the laboratory at the University of Pennsylvania during the

seven consecutive study nights, and were allowed to return home during the day for routine activities between nights.

Subjects were run in batches of four participants at a time. At night, polysomnographic recordings were scored for factors such as awakenings and sleep staging (see Results). During the morning after awakening, subjects underwent a series of assessments involving blood pressure measurements, 5-min rested heart rate variability measurements, driving simulator assessments, self-reported survey assessments (e.g., sleep quality), and a neurobehavioral performance test battery. Assessments are further described in the 2023 FAA ASCENT Project 86 annual report (Basner, 2023).

2.2. Sample Collection and Analyses

Following a night of experimental noise (or control) exposure, a 2.5 mL blood sample was collected in the morning into a PAXgene® RNA tube (BD 762165) to preserve the RNA. Immediately following collection, the tube contents were mixed by gently inverting the tube by hand ten times, and incubated at room temperature for approximately 2–6 hours. Tubes next underwent a stepdown freezing protocol beginning with approximately 24 hours at -20 degrees Celsius, and then were transferred to -80 degrees Celsius until RNA extraction. The extraction of RNA, library preparation, and total RNA sequencing (RNASeq) were conducted at the Baylor College of Medicine Human Genome Sequencing Center. Total RNA extractions were conducted using the Chemagic Prime Total RNA Blood 4k kit (PerkinElmer, catalog #CMG-1484) and the Magnetic Bead technology Chemagic Prime 8 platform. Library preparation was conducted with the Illumina TruSeq Stranded Total RNA with Ribo-Zero Globin kit, followed by total RNA sequencing to generate a target threshold of at least 100 million reads per sample of 150 base paired-end reads as previously described (Uyhelji et al., 2023).

Sequence reads underwent pipeline processing in the Amazon cloud on Linux platforms, facilitated by the Department of Transportation Secure Data Commons. Briefly, sequence reads were visually inspected with FastQC (Andrews, 2010) and trimmed with CutAdapt v4.3 to eliminate adapters from the reads, discard reads shorter than 50 bases, and remove low-quality bases with the flag --nextseq-trim=20 (Martin, 2011). Next, reads were mapped to the human T2T-CHM13v2.0 reference genome (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_009914755.1). Mapping consisted of paired-read alignment using STAR v.2.7.10b using the –outMultimapperOrder Random flag for random output of multimapping reads (Dobin et al., 2012) with a length of 150. Gene expression count data were generated from mapped reads using featureCounts v2.0.5 set for strand-specific paired-read usage with a minimum read length of 50 bases, and discarding chimeric fragments aligned to distinct chromosomes (Liao et al., 2013).

Linear models were constructed from the resulting gene count data. A total of 151 samples were collected from the morning following each exposure night, excluding the first acclimation night. Samples lacking associated phenotype information were removed prior to normalization and construction of genetic models (**Supplementary Online Table 1**), resulting in a final analysis of 25 subjects with successful molecular and phenotypic data collection. For

analysis of gene expression related to polysomnography, due to equipment failure and issues during data collection, additional samples had to be removed based on insufficient information.

Results from the remaining samples (**Supplementary Online Table 1**) were analyzed in CRAN R versions 4.4.1-3. (R Core Team, 2024) using limma v. 3.60.4-3.62.2 (Ritchie et al., 2015). First data were filtered for low expression and normalized using the trimmed mean of M values approach (Robinson & Oshlack, 2010) prior to linear modeling with the voom approach (Law et al., 2014). Specifically, the function voomLmFit was used, and P values were corrected for multiple testing using the Benjamini and Hochberg method to yield a False Discovery Rate (FDR). Genes were considered significantly differentially expressed for a factor of interest if linear models resulted in a FDR below 0.05. The subject was modeled as a random effect. Principal component analysis indicated a clear separation of libraries generated from biological males and females; thus biological sex was incorporated as a fixed binary cofactor model term. Other model fixed effect terms consisted of the treatment condition group (cofactor designating status as a night of CTRL, AN, AN+EP, AN+BN40, AN+BN50, or BN50 exposure) and a covariate term to indicate the numeric value for the phenotypic assessments (e.g., blood pressure, driving simulator, neurobehavioral performance, or polysomnography metric). To test for noise exposure and mitigation countermeasure effects, additional tests were run among specific exposure conditions, omitting the phenotypic covariate and testing for genes differentially expressed relative to the exposure conditions. Finally, select lists of differentially expressed genes were submitted to QIAGEN Ingenuity Pathway Analysis (Krämer et al., 2014), as described in (Uyhelji et al., 2023)*.*

3. Results and Discussion

Linear models testing for a relation between gene expression and treatment exposure conditions, based on data encompassing all six conditions, revealed 1,246 differentially expressed genes (FDR<0.05) (**Table 1, Supplementary Online Table 2**). Subsequent model runs were conducted, repeating the analysis on subsets of the data to investigate which of the conditions may have contributed to the molecular response. For instance, to test BN50 compared to CTRL, only nights with no noise (control) and nights with pink noise at 50 dBA were included. Based on these model runs limited to specific exposure conditions, the only comparison yielding differentially expressed genes was that of the control (no noise) compared to aircraft noise exposure plus pink noise at 40 dBA (**Table 1, Supplementary Online Table 3)**. It is not known why this comparison and not control versus aircraft noise exposure plus pink noise at 50 dBA yielded differential gene expression. An additional model run of just the aircraft noise (no mitigation) and control exposures was tested, after limiting the differentially expressed genes to the 3,719 genes identified as differentially expressed relative to time awake in a previous study of total sleep deprivation (Uyhelji et al., 2023). This additional analysis was performed to limit the number of tested genes and thereby reduce the stringency of the FDR Benjamini Hochberg multiple testing correction. Yet even with this approach, there were no genes detected as differentially expressed between simulated aircraft noise and the quiet control exposure.

A small number of genes were differentially expressed relative to physiology and performance metrics (**Tables 2**) based on separate model runs, drawing on data across all study nights (all condition exposures). Rather than testing for an exposure effect, these model runs tested for an effect of the metric of interest. These models yielded genes differentially expressed relative to blood pressure and heart rate variability metrics, driving simulator performance, and speed across the Cognition battery (Basner et al., 2020; Basner et al., 2015). Of 15 different sleep metrics assessed from polysomnography recordings of nighttime sleep, only PSG_nAWRperhTST_Noise was associated with differential gene expression. This variable is defined as the number of awakenings per hour of sleep [noise periods only], and was associated with the differential expression of 2,181 genes (**Table 3, Supplementary Online Table 4**).

Table 1

Differential gene expression relative to condition exposure comparisons. Each row represents a distinct model run, from data collected on nights reflecting the condition exposures noted, testing for an effect of exposure.

Condition Exposures	Number of Differentially Expressed Genes
All	1,246
AN vs. CTRL	0
AN, AN+BN50, and AN+BN40	0
AN+EP vs. AN	0
BN50 vs. CTRL	0
CTRL vs. Noise (where noise was defined as any of the conditions except CTRL)	0
CTRL vs. AN+BN40	1,306
CTRL vs. AN+BN50	0
AN vs. AN+BN40	0

Table 2

Differential gene expression relative to physiology and performance metrics. Each row represents a distinct model run, from data collected across nights reflecting all condition exposures, testing for an effect of the physiology or performance metric noted.

Physiology and Performance Metric	Number of Differentially Expressed Genes	Symbols for Differentially Expressed Genes
Accuracy	0	
BP_Av_Dia	2	*TRDV2, RAB6C*

Physiology and Performance Metric	Number of Differentially Expressed Genes	Symbols for Differentially Expressed Genes
BP_Av_Sys	1	*TRDV2*
DS_DA_AvRT	5	*HERC2P3, B4GALNT3, ZNF890P, LINC02937, C14orf132*
DS_SDLanePos	6	*MGST2, NHIP, ETV7, USP32P2, HERC2P3, BPI*
Hfpow_FFT_ms_sq	6	*PDK2, PIGN, PLGLB1, FCGR3B, HLA-A, PLOD2*
HRV_SDNN_ms	4	*PDK2, PLGLB1, DNAJC15, CCDC146*
MS_KSS	0	
MS_POMS_Total	0	
PVT_Lapses	0	
Speed	3	*CLEC9A, MYOM2, BTNL3*
SU_PoorSleepQual	0	

Table 3

Differential gene expression relative to scored metrics from polysomnography assessments of sleep. Each row represents a distinct model run, from data collected across nights reflecting all condition exposures, testing for an effect of the polysomnography metric noted.

Polysomnography Metric	Number of Differentially Expressed Genes
PSG_N1	0
PSG_N2	0
PSG_N3	0
PSG_N3REM	0
PSG_nAWRperhTST	0
PSG_nAWRperhTST_Noise	2,181
PSG_REM	0
PSG_REML	0
PSG_SE	0
PSG_SOL	0
PSG_Wake	0
ORP_Av_All	0
ORP_Av_All_Noise	0
PSG_nAROperhTST	0
PSG_nAROperhTST_Noise	0

Functional analyses made with QIAGEN Ingenuity Pathway Analysis Core Analysis are consistent with an impact of simulated aircraft noise, indicating pink noise at 40 dBA impacted pathways related to translation, i.e., protein synthesis. Of the top five canonical pathways based on the 1,306 genes differentially expressed between CTRL and AN+BN40 exposure conditions, one was related to Eukaryotic Translation Elongation (P=1.47E-44), one to Eukaryotic Translation Termination (P=2.13E-41), and one to Eukaryotic Translation Initiation (P=5.24E-40). The other two pathways consisted of SRP-Dependent Cotranslational Protein Targeting to Membrane (P=3.38E-44), and the Response of EIF2AK4 (GCN2) to Amino Acid Deficiency, with *EIF2AK4* being the gene symbol for *Eukaryotic Translation Initiation Factor 2 Alpha Kinase 4*. These pathways are predicted to be upregulated in the exposure condition of aviation with pink noise (AN+BN40) relative to the control exposure. Research has suggested a positive association of protein synthesis with sleep, particularly Non-Rapid Eye Movement sleep, as reviewed in (Grønli et al., 2014). Prior research also has identified downregulation of the gene *Eukaryotic Translation Initiation Factor 4E Family Member 3* (*EIF4E3*) in association with human total sleep deprivation (Uyhelji et al., 2018). Hence the upregulation observed here might suggest a beneficial role of the use of 40 dBA pink noise during aviation noise toward improving sleep and protein synthesis, but it is not clear why this effect is observed in the comparison of AN+BN40 to CTRL and not AN to AN+BN40. Further work is needed to explore the implications for human health and performance from the observed molecular changes.

Because only a handful of genes were differentially expressed relative to blood pressure, heart rate, driving simulator, and speed performance metrics (**Table 2**), no pathway analysis was conducted for these gene lists. However, QIAGEN Ingenuity Pathway Analysis Core Analysis of the 2,181 genes differentially expressed relative to the polysomnography metric PSG_nAWRperhTST_Noise was performed and yielded functional inferences. Pathway analysis indicated a positive association of the number of awakenings with upregulation of the Generic Transcription Pathway (P=1.91E-09). Results also suggested the potential for a molecular stress response, with two of the top canonical pathways being the Protein Ubiquitination Pathway (P=2.23E-04) and the Unfolded Protein Response (P=5.25E-04), both tending toward upregulation. As reviewed by (Naidoo, 2009), the ubiquitin-proteasome system can serve to eliminate misfolded or damaged proteins, and the unfolded protein response plays a cytoprotective role in mitigating the harmful effects of sleep loss. Hence these findings may indicate that the awakenings from noise exposure resulted in a molecular stress response.

4. Conclusion

Analyses of gene expression changes did not reveal a notable impact of simulated aircraft noise as compared to a control night without noise exposure, with zero genes differentially expressed between these two conditions. Of the noise exposures tested, only simulated aircraft noise with the addition of 40 dBA pink noise as compared to control yielded differential gene expression. Functional analysis suggests this noise condition may be associated with the induction of pathways involved in protein translation. Further research is needed to decipher the reason this particular condition (and not aircraft noise without mitigation compared to aircraft noise with 40 dBA pink noise) yielded an impact on gene expression. Additional work is also required to understand the absence of a response in analyses with 50 dBA pink noise.

Repeated testing with a larger sample size may be worthwhile to increase confidence that simulated aircraft noise by itself does not impact gene expression. Yet the present findings may indicate that, given subjects were provided with an 8 hour sleep opportunity, the noise exposure used in this study did not lead to severe enough sleep disruption to have a discernable molecular impact. Only a handful of genes changed in association with performance, blood pressure, and heart rate variability assessments across all nights of exposure. In contrast, a large number of genes changed in association with awakenings during noise events as monitored with polysomnography, which could indicate that molecular effects are only pronounced when noises are sufficient to cause a participant to awaken from sleep. These differentially expressed genes may be considered candidate biomarkers (biological indicators) of awakenings during noise events, and if validated with additional research, may be useful as a complementary sample-based approach to enhancing the monitoring of sleep disruption. Absence of a large molecular response in association with neurobehavioral performance metrics such as Psychomotor Vigilance Test lapses may indicate the sleep disruption was too mild to yield a significant cognitive effect.

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6. Appendix A. List of Supplementary Online Tables

6.1. Appendix A Second Level

Supplementary Online Table 1
*List of samples analyzed in tests of genes relative to both exposure condition (**Table 1**) and to physiology and performance (**Table 2**), as well as the subset tested relative to polysomnographic metrics (**Table 3**). Samples were removed from analyses due to missing or unreliable information for one or more phenotypic variables. For polysomnography metrics two sets of samples were used. Based on four metrics (PSG_nAROperhTST, ORP_Av_All, ORP_Av_All_Noise, and PSG_nAROperhTST_Noise) that were missing data at additional timepoints, they were tested with a reduced sample set indicated as "Polysomnography Subset".*

Supplementary Online Table 2
Differential expression of 1,246 genes (FDR<0.05) relative to exposure condition, in an overall test of all six exposures: CTRL, AN, AN+BN40, AN+BN50, AN+EP, BN50.

Supplementary Online Table 3
Differential expression of 1,306 genes (FDR<0.05) in the AN+BN40 condition exposure relative to CTRL. Positive values for log fold change (logFC) indicate higher expression in AN+BN40.

Supplementary Online Table 4
Differential expression of 2,181 genes (FDR<0.05) relative to PSG_nAWRperhTST_Noise as measured across all six exposure condition nights. Positive values for log fold change (logFC) indicate a positive association of gene expression with higher values for PSG_nAWRperhTST_Noise.