Brain activity and transcriptional profiling in mice under chronic jet lag

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Shift work is known to be associated with an increased risk of neurological and psychiatric diseases, but how it contributes to the development of these diseases remains unclear. Chronic jet lag (CJL) induced by shifting light-dark cycles repeatedly is a commonly used protocol to mimic the environmental light/dark changes encountered by shift workers. Here we subjected wildtype mice to CJL and performed positron emission tomography imaging of glucose metabolism to monitor brain activities. We also conducted RNA sequencing using prefrontal cortex and nucleus accumbens tissues from these animals, which are brain regions strongly implicated in the pathology of various neurological and psychiatric conditions. Our results reveal the alterations of brain activities and systematic reprogramming of gene expression in brain tissues under CJL, building hypothesis for how CJL increases the susceptibility to neurological and psychiatric diseases.

Background & Summary

Approximately 15%–30% of the working population worldwide are engaged in some type of shift work1. Based on epidemiological surveys, shift work is associated with numerous adverse health outcomes such as type 2 diabetes, cancer, cardiovascular disorders and immune dysfunction2-4. Shift work is also known to increase the risk of neurological diseases. To be specific, shift work may trigger migraine, exacerbate epilepsy and increase dementia incidence5-6. In addition, shift work is associated with cognitive deficits7-9, sleep disorders10,11, mood alteration and increases the risk of mental illnesses including depression, anxiety, alcohol abuse, and schizophrenia12-15. Individuals undergoing shift work experience alternating light/dark cycles for extended time and suffer from circadian disruptions due to the misalignment between endogenous rhythm and external time. To mimic shift work condition, a number of studies used rodents subjected to chronic jet lag (CJL) by continuously shifting the timing of light-dark cycles and found that CJL promotes tumour progression16-18, obesity19, addiction20, and impairs innate immune responses21. Recent work in rodents also demonstrated that CJL leads to phenotypes related to mood disorders, but the mechanism is largely unknown22,23.

Glucose turnover rate serves as an indicator of brain activities, while alterations of brain activities are linked to many neurological and psychiatric diseases24. Abnormal glucose metabolism in the brain has been reported in dementia, epilepsy, major depressive disorder, and bipolar disorder25-27. The prefrontal cortex (PFC) is known to be involved in regulating a number of cognitive and emotional processes28. Dysfunction of the PFC has been found in various psychiatric and neurological disorders, including depression, anxiety disorders, addiction, schizophrenia, autism spectrum disorders, Alzheimer's disease, and Parkinson's disease29-32. Nucleus accumbens (NAc) plays a central role in processing motivation, reward and aversion33-35. NAc has been implicated in the pathophysiology and treatment of mental illnesses such as major depressive disorder, addictive disorders, schizophrenia, obsessive-compulsive disorder and anorexia nervosa36-39.

In this study, we subjected wildtype mice to CJL following a previously reported protocol40 (Fig. 1). Total sleep duration is not reduced by CJL, indicating that the physiological and molecular changes we report here are not due to deficient sleep caused by CJL (Fig. 2). We employed positron emission tomography (PET) to monitor glucose uptake in the brain of mice maintained under baseline condition or CJL for slightly over a month, as it has previously been shown that CJL treatment for about a month can elicit prominent changes in metabolic

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indices and lung mechanics.\textsuperscript{40,41} We present raw PET images as well as quantified data collected on Day 34 of CJL treatment, which demonstrate that CJL significantly reduces activities throughout the brain (Fig. 3). Given the prominent and pervasive alterations in brain activity observed by Day 34 of CJL, we reasoned that molecular changes contributing to this should start to occur at an earlier stage of CJL treatment. It has been reported that metabolic phenotypes start to become significant by Day 10 of CJL, and ten days of CJL is sufficient to induce substantial effects on the expression of a number of circadian genes.\textsuperscript{40,42} Therefore, we conducted RNA sequencing (RNA-seq) on PFC and NAc harvested from animals during the light and dark phase under baseline condition and on Day 10 of CJL treatment, respectively. These data will provide valuable information for studies that investigate how shift work or circadian disruptions increase the risk of disorders associated with the brain.

Methods

Animals and chronic jet lag treatment. 8-week-old male C57BL/6J mice purchased from Model Animal Research Center of Nanjing University were assigned to different cages randomly with approximately 5 animals per cage. Animals were housed in light-tight cabinets with time-controlled illumination. Food and sterilized water were accessible ad libitum. The mice were housed under 12 hour light: 12 hour dark (12L12D) for 2 weeks for entrainment prior to CJL treatment. The experimental design is displayed in Fig. 1. For CJL group, lights on and off times were advanced by 6 hours every 2 days following a previously published protocol, whereas for control group they remain unchanged.\textsuperscript{40,43} Nine mice were subjected to PET imaging on Day 34 of CJL (the last jetlag event before PET imaging occurred on Day 32) along with six controls. The brain tissues of six mice (three per time point) were harvested for RNA-seq on Day 10 of CJL (the last jetlag event before tissue collection occurred on Day 8) along with equal number of controls. To assess sleep, seven mice were individually housed and sleep monitoring was conducted on Day 8, 18, and 28 of CJL. All mouse work was performed in accordance with the guidelines of Institutional Animal Care and Use Committee at Huazhong University of Science and Technology.

Sleep monitoring. Sleep was monitored by PiezoSleep Mouse Behaviour Tracking System as described earlier.\textsuperscript{44} Briefly, seven animals were individually housed in a cage with piezoelectric film sensor underneath that transmitted activity signals (motions and breathing movements) to monitoring software (PiezoSleep 2.18, Signal Solutions). Sleep can be distinguished from wakefulness by rhythmic signals (approximately 3 Hz) generated by typical respiration patterns during sleep.\textsuperscript{44} The baseline pressure signal from the piezoelectric sensors was sampled at 120 Hz. Features associated with sleep and wake behaviors were extracted from the pressure signals and saved every 2 seconds. Sleep-wake classification was performed automatically based on decision statistics.\textsuperscript{45} Data collected were binned over 1 hour using the average of percent sleep (average percent of time in sleep state). Data were analyzed with SleepStats and exported as hourly percent sleep, hourly sleep bout number and hourly average sleep bout length of the specified day. Sleep bout was computed as the duration of an interval of continuous sleep. The minimum sleep bout length parameter was set to 30 s by default, which means a bout length count was initiated only when a 30 s-interval contained greater than 50% sleep.\textsuperscript{40,47} Setting the minimum bout length as 30 s
can eliminate the impact of short and ambiguous arousals on computing bout length and reduce the probability of error46. Based on the exported data, average percent sleep, sleep bout number, and average length of sleep bout of a specified day were calculated.

PET. 18F-fluorodeoxyglucose (18F-FDG) is the most commonly used marker for glucose metabolism in PET imaging48. Nine male C57BL6/J mice subjected to CJL and six control mice were used. Mice were fasted during the dark phase for 12 h before the test. Prior to PET imaging, animals were injected with (250 ± 10 μCi) 18F-FDG intraperitoneally. After one hour, they were anesthetized with 2% isoflurane and imaging was conducted from Zeitgeber Time 1 (ZT1, 1 hour after lights on; ZT0 is defined as the time of lights on) to ZT6. Images of mouse brains were obtained with the static scanning pattern (10 min) by Trans-PET® BioCaliburn® LH (Raycan Technology, China), a PET system for imaging small animals49. The PET images were reconstructed using three-dimensional OSEM method with a voxel size of 0.5 × 0.5 × 0.5 mm3. Glucose uptake was measured as the mean standardized uptake value (SUV).

Brain dissection and RNA extraction. Brain tissues of three CJL treated animals and three control animals fed ad libitum were harvested at ZT1 and ZT13, respectively. After cervical dislocation, the skin was flipped over the eyes to free the skull. Then the skull was broken and brain was removed gently out of the skull. The brain was immediately transferred to a Petri dish chilled on ice and placed with ventral surface facing up. The brains of mice were dissected according to previously published protocols50–53. Coronal sections were made from the rostral end of the brain using a sharp and chilled razor blade. PFC, the anterior part of the brain just behind the olfactory bulb, was removed from the first ~1.5 mm-thick coronal section50,51. NAc was located in the subsequent section (~1.0 mm-thick) and was identified based on the location of anterior commissure with the approximate anterior-posterior coordinate of +1.8 mm ~0.6 mm from Bregma52,53. The dissected PFC and NAc tissues were immediately transferred to liquid nitrogen and stored at −80 °C for further processing.

Total RNA was extracted using RNA isolater (Vazyme, China). RNA integrity was assessed with RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Alilent Technologies, USA) before the samples were sent for RNA-seq.

Library preparation, RNA sequencing and data processing. The sequencing libraries were generated using VAHTS mRNA-seq v2 Library Prep Kit for Illumina® (Vazyme, NR601) following manufacturer’s recommendations. Briefly, mRNA was purified and fragmented and cDNA was synthesized with the mRNA fragments.
as templates. Then the cDNAs were ligated with special sequencing adaptor and the products with appropriate size were selected for PCR. After passing quality examination, the generated libraries were sequenced on Illumina Hiseq X Ten platform with 150 bp paired-end module.

The raw reads were filtered to produce clean data by excluding sequencing adaptors and low quality reads, including reads containing over 5% “N” and those containing >50% bases with quality value less than 10. The quality assessment of clean data was performed using FastQC v0.11.9.

Sequencing reads were then aligned to the mouse mm10 reference genome using HISAT2. The differential expression analysis was performed by DESeq. 2 with default parameters. The significance threshold of \( P < 0.05 \) was applied. The number of differentially expressed genes (DEGs) and the number of genes that exhibited a fold-change >2 in each brain tissue at each time point were displayed in Table 1.

### Data Records

Sequencing data and the list of differentially expressed genes can be accessed at NCBI Gene Expression Omnibus (GEO) with the accession number GSE15354054. The PET data and images have been deposited in Figshare55.

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**Table 1.** Number of DEGs \((P < 0.05)\) and genes that exhibit more than two-fold change \((FC > 2 \text{ or } <0.5)\). Ctrl, control; CJL, chronic jet lag.

| Samples | No. of DEGs | No. of up-regulated genes | No. of down-regulated genes | No. of genes with \(P < 0.05 \text{ \& FC} > 2\) | No. of genes with \(P < 0.05 \text{ \& FC} < 0.5\) |
|----------|-------------|--------------------------|-----------------------------|---------------------------------|---------------------------------|
| PFC ZT1 Ctl vs Ctrl | 3088 | 1535 | 1553 | 194 | 240 |
| PFC ZT13 Ctl vs Ctrl | 2020 | 1189 | 831 | 139 | 204 |
| Ctrl ZT13 vs ZT1 | 4699 | 2211 | 2488 | 278 | 304 |
| CJL ZT13 vs ZT1 | 1221 | 573 | 648 | 162 | 253 |

**Table 2.** Statistics of sequencing data. Clean data rate \(=100% \times \text{Clean reads/Raw reads. Ctrl, control; CJL, chronic jet lag.}

| Samples | Raw reads | Clean reads | Clean read rate (%) | GC (%) | mapping rate (%) | Q20 (%) | Q30 (%) | Accession |
|---------|-----------|-------------|---------------------|--------|-----------------|---------|---------|-----------|
| PFC ZT1 Ctl_1 | 59674380 | 59285246 | 99.35 | 48.75 | 96.35 | 97.28 | 93.32 | GSM4646833 |
| PFC ZT1 Ctl_2 | 59262454 | 58394418 | 98.54 | 48.36 | 96.91 | 97.63 | 93.98 | GSM4646834 |
| PFC ZT1 Ctl_3 | 53675490 | 53181490 | 99.11 | 48.65 | 96.54 | 97.33 | 93.41 | GSM4646835 |
| PFC ZT13 Ctl_1 | 52488284 | 52184492 | 99.42 | 49.18 | 96.32 | 97.12 | 92.99 | GSM4646836 |
| PFC ZT13 Ctl_2 | 54765670 | 54529024 | 99.57 | 48.81 | 96.74 | 97.06 | 92.89 | GSM4646837 |
| PFC ZT13 Ctl_3 | 51643852 | 51358972 | 99.45 | 48.89 | 96.50 | 96.97 | 92.72 | GSM4646838 |
| PFC ZT1 Ctl_1 | 55064574 | 54814420 | 99.55 | 49.06 | 96.65 | 97.09 | 92.94 | GSM4646839 |
| PFC ZT1 Ctl_2 | 56191198 | 55940824 | 99.55 | 49.09 | 96.77 | 97.00 | 92.77 | GSM4646840 |
| PFC ZT1 Ctl_3 | 55392450 | 55117524 | 99.50 | 49.34 | 96.70 | 97.18 | 93.09 | GSM4646841 |
| PFC ZT13 Ctl_1 | 5479172 | 5225860 | 99.54 | 49.06 | 96.80 | 97.19 | 93.14 | GSM4646842 |
| PFC ZT13 Ctl_2 | 5733648 | 5449378 | 99.45 | 48.84 | 96.78 | 97.17 | 93.1 | GSM4646843 |
| PFC ZT13 Ctl_3 | 5460704 | 54328066 | 99.48 | 49.08 | 96.63 | 97.25 | 93.27 | GSM4646844 |
| NAc ZT1 Ctl_1 | 52250318 | 52046014 | 99.61 | 48.57 | 93.60 | 94.72 | 88.17 | GSM4646845 |
| NAc ZT1 Ctl_2 | 46872158 | 46551084 | 99.32 | 48.18 | 94.76 | 95.58 | 89.94 | GSM4646846 |
| NAc ZT1 Ctl_3 | 47492160 | 47253747 | 99.59 | 48.94 | 94.27 | 95.32 | 89.42 | GSM4646847 |

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as templates. Then the cDNAs were ligated with special sequencing adaptor and the products with appropriate size were selected for PCR. After passing quality examination, the generated libraries were sequenced on Illumina Hiseq X Ten platform with 150 bp paired-end module.

The raw reads were filtered to produce clean data by excluding sequencing adaptors and low quality reads, including reads containing over 5% “N” and those containing >50% bases with quality value less than 10. The quality assessment of clean data was performed using FastQC v0.11.9.

Sequencing reads were then aligned to the mouse mm10 reference genome using HISAT2. The differential expression analysis was performed by DESeq. 2 with default parameters. The significance threshold of \( P < 0.05 \) was applied. The number of differentially expressed genes (DEGs) and the number of genes that exhibited a fold-change >2 in each brain tissue at each time point were displayed in Table 1.

**Data Records**

Sequencing data and the list of differentially expressed genes can be accessed at NCBI Gene Expression Omnibus (GEO) with the accession number GSE15354054. The PET data and images have been deposited in Figshare55.
A high percentage of clean reads from each sample was acquired after filtering the raw reads. The statistics summary of clean reads is presented in Table 2. All samples produced more than 98.5% clean reads and >93.60% of the clean data were mapped to the reference genome. In addition, GC content, Q20 and Q30 were also calculated. For all samples, the GC content was stable with distributed range from 48.36% to 49.18%. The read base quality was assessed by FastQC and displayed in Fig. 4. Most of the base quality scores were above 30, suggesting high-quality RNA-seq data.

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Author contributions
L.Z. initiated the project and oversaw all aspects of the project. Q.G. and S.K. performed the experiments. Q.G. carried out the data analysis. Q.G. and L.Z. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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