



Diurnal rhythms across the human dorsal and ventral striatum

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Funding:
• R01MH11601 (Colleen McClung)
• NARSAD Independent Investigator Award (Colleen McClung)
• 5T32MH016804 (Robert Sweet)
• F32MH120907 (Kyle Ketchesin)

INTRODUCTION

- Circadian rhythms are natural, internal processes that regulate many metabolic processes and repeat roughly every 24 hours.
 - These rhythms in cellular function are primarily governed by a core molecular transcriptional/translational feedback loop consisting of core clock genes such as CLOCK, NPAS2 etc.
 - Our previous studies on cortical regions found
 - Many of the same transcripts are rhythmic across prefrontal cortical regions with very similar phase.
 - Subjects with psychiatry diseases or drug addiction have different rhythmic pattern in transcripts compared with control subjects.
 - In humans, the striatum has been subdivided into three main regions that serve some overlapping and some distinct functions:
 - Nucleus accumbens (NAc): reward and motivation
 - Caudate: cognition and working memory
 - Putamen: motor control
- A sequential chain of events to produce goal-directed behavior, beginning with motivation for the goal, cognitive processing, and finally motor response, which is closely related to mental disorders.

RESEARCH GOAL

- Investigate diurnal rhythms in gene expression within each of the three regions in subjects without neurological disorders.
- Compare across brain regions to identify similarities and differences in terms of both functions (pathway analysis) and circadian patterns.

Future work: Determine whether the healthy gene expression rhythms established from this project are altered in a cohort of subjects with schizophrenia or bipolar disorder.

METHODS

Data preparation

- Postmortem NAc, caudate, and putamen tissue samples from control, schizophrenia, and bipolar disorder subjects were obtained through the University of Pittsburgh Brain Tissue Donation Program and the NIH NeuroBioBank.
- We generated a control cohort (n=59) based on three criteria: 1) known time of death (TOD) within a 4-hour window and meeting the criteria of rapid death; 2) age less than 65 years; 3) postmortem interval (PMI) less than 30 hours.
- Normalize TOD for each subject to a zeitgeber (ZT) scale.
 - TOD was first converted to coordinated universal time (UTC) followed by adjustment with longitude, latitude, and elevation of death place.
 - Each subject's TOD was set as ZT = t hours after previous sunrise (if t < 18) or before next sunrise (if t ≥ -6).

Statistical Analysis

Nonlinear regression was used to detect circadian patterns of transcript expression based on individual TOD.

$$\begin{matrix} s_1, s_2, \dots, s_n \\ \vdots \\ g_1 \\ g_2 \\ \vdots \\ g_G \end{matrix} \text{Gene expression matrix} \xrightarrow{\text{expr}_{g_i}} \begin{matrix} \hat{A}, \hat{p}, \hat{\sigma}, \hat{R}^2 \\ \vdots \\ g_1 \\ \vdots \\ g_G \end{matrix} \text{Fitted parameters}$$

$$\text{Gene expression matrix} = A_g \sin\left(\frac{2\pi}{24}(TOD_i + p_g)\right) + o_g + N(0, \sigma)$$

Where $R^2 = 1 - RSS_M / RSS_0$. Then p-values of R^2 were derived from permutating samples and re-fit this sinusoidal curve for each permutation followed by Benjamini-Hochberg correction.

RESULTS

Circadian rhythm analysis in each striatal region

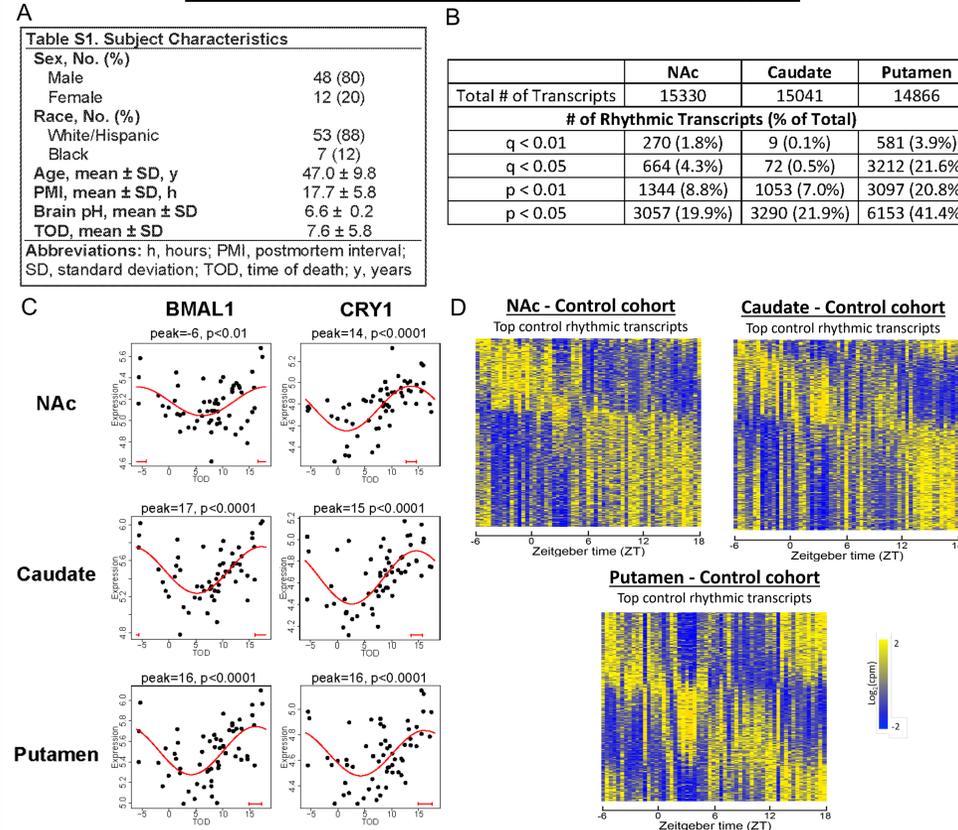


Figure 1: (A) Characteristics of the control cohort (n=59). (B) The number of rhythmic transcripts in each striatal region. (C) Scatterplots of two core circadian clock genes, BMAL1 and CRY1 (x-axis: TOD on ZT scale, y-axis: gene expression level) with fitted sinusoidal curves (red). (D) Heatmaps depicting the top rhythmic transcripts (p < 0.01). Row: transcripts ordered by their peak times. Column: subjects ordered by TOD.

Comparison of top rhythmic genes shows both similarities and differences across striatal regions

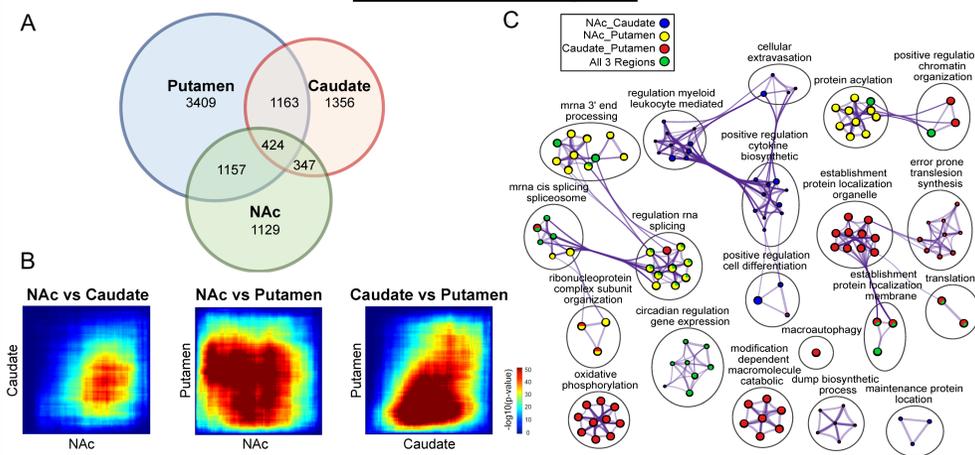


Figure 2: (A) Venn diagrams showing overlap of rhythmic genes at p < 0.05. (B) RRHO plot comparing gene ranks between each pair of striatal regions: A high degree of overlap in rhythmic genes between the NAc and putamen and the caudate and putamen, but a small degree of overlap between the NAc and caudate. (C) GO Biological Process enrichment via Metascape showing different functions for genes rhythmic in more than 2 regions: Genes rhythmic in all 3 regions / the NAc and caudate only / the NAc and putamen only / the caudate and putamen only are enriched in circadian rhythms and mRNA processing / immune function / energy metabolism / mitochondrial function respectively.

RESULTS (cont.)

Phase shift between the NAc and caudate and the caudate and putamen suggests a temporal wave of transcript expression in specific cellular processes across the striatum.

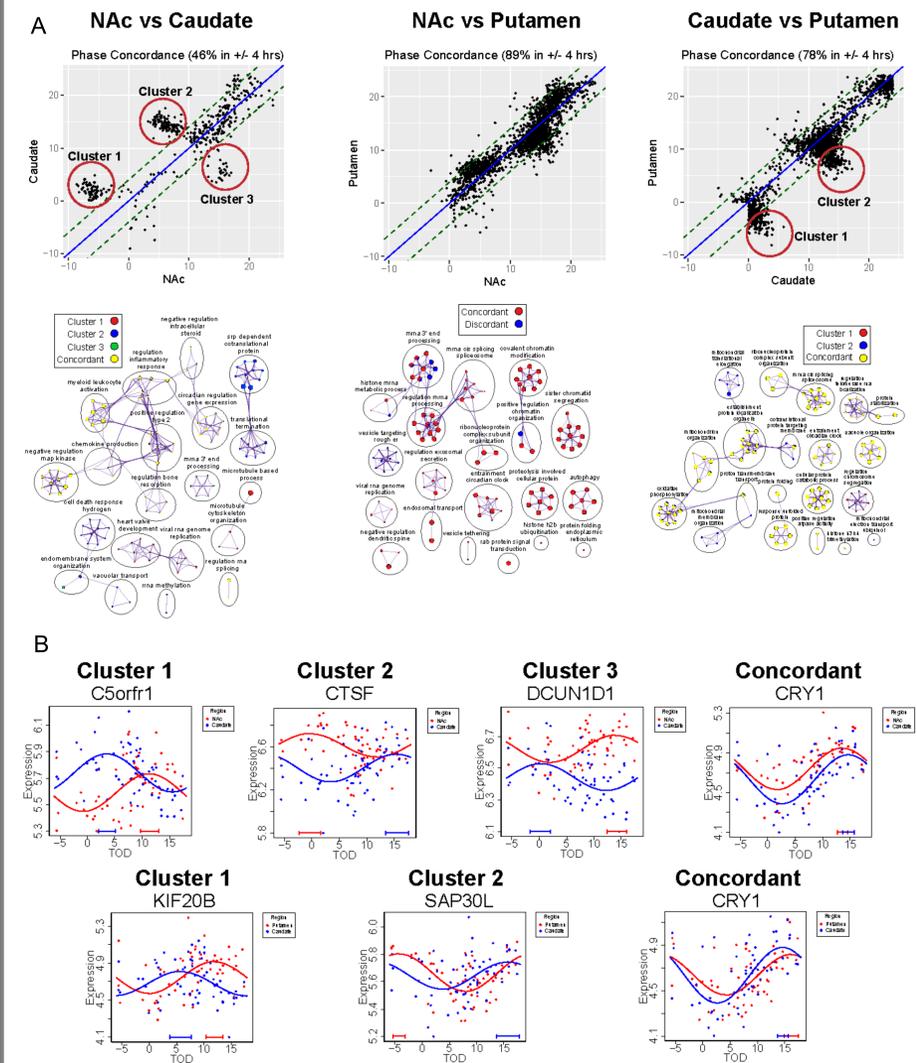


Figure 3: (A top) Phase concordance plots showing the phase relationship in common rhythmic transcripts between each pair of the regions. For a given transcript, phases were plotted between the two regions and was considered concordant if their phase differences fell within a window of +/- 4 hours (dash line). (A bottom) GO Biological Process enrichment via Metascape for concordant and discordant transcripts between regions. (B) Double-plotted scatterplots for NAc and caudate (top) and caudate and putamen (bottom) with bars depicting 90% confidence intervals of peak estimates.

CONCLUSIONS

- Core circadian clock genes are rhythmic across all three regions and show strong phase concordance across regions. However, the putamen contains a much larger number of significantly rhythmic transcripts.
- Rhythmic genes across different regions show different mechanisms.
- NAc and putamen are generally in phase with regards to timing of expression rhythms while the NAc and caudate, and caudate and putamen, have several clusters of discordant rhythmic transcripts, suggesting a temporal wave of specific cellular processes across the striatum.