

# Astrocyte Molecular Clock Function in the Nucleus Accumbens Is Important for Reward-Related Behavior

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## ABSTRACT

**BACKGROUND:** Substance use disorders are associated with disruptions in circadian rhythms. Both human and animal work have shown the integral role for circadian clocks in the modulation of reward behaviors. Astrocytes have emerged as key regulators of circadian rhythmicity. However, no studies to date have identified the role of circadian astrocyte function in the nucleus accumbens (NAc), a hub for reward regulation, or determined the importance of these rhythms for reward-related behavior.

**METHODS:** Using astrocyte-specific RNA sequencing across time of day, we first characterized diurnal variation of the NAc astrocyte transcriptome. We then investigated the functional significance of this circadian regulation through viral-mediated disruption of molecular clock function in NAc astrocytes, followed by assessment of reward-related behaviors, metabolic-related molecular assays, and whole-cell electrophysiology in the NAc.

**RESULTS:** Strikingly, approximately 43% of the astrocyte transcriptome has a diurnal rhythm, and key metabolic pathways were enriched among the top rhythmic genes. Moreover, mice with a viral-mediated loss of molecular clock function in NAc astrocytes show a significant increase in locomotor response to novelty, exploratory drive, operant food self-administration, and motivation. At the molecular level, these animals also show disrupted metabolic gene expression, along with significant downregulation of both lactate and glutathione levels in the NAc. Loss of NAc astrocyte clock function also significantly altered glutamatergic signaling onto neighboring medium spiny neurons, alongside upregulated glutamate-related gene expression.

**CONCLUSIONS:** Taken together, these findings demonstrate a novel role for astrocyte circadian molecular clock function in the regulation of the NAc and reward-related behaviors.

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Substance use disorders (SUDs) remain a devastating public health issue in the United States (1,2) that lacks effective therapeutics (3). A potential treatment target may involve circadian rhythms. While it has long been known that individuals with SUDs have disrupted circadian and sleep/wake rhythms (4,5), there is a growing body of evidence suggesting a bidirectional relationship between circadian rhythm disruption and substance abuse. Both human and animal studies indicate that disruptions to circadian rhythms and sleep/wake may increase SUD vulnerability (6). Specifically, changes at the molecular level of the circadian system may play an essential role in the establishment and/or reinforcement of drug addiction. In turn, exposure to drugs of abuse further disrupts molecular clock function, resulting in a self-perpetuating cycle.

In mammals, circadian rhythms are controlled by a series of transcription-translation feedback loops that cycle every 24 hours (7). This molecular clock consists of the core circadian transcription factors CLOCK or NPAS2, which form a complex with BMAL1 and drive expression of the proteins PER and

CRY. PER and CRY heterodimerize in the cytosol, translocate into the nucleus, and interact with CLOCK/NPAS2:BMAL1 to inhibit their own transcription. This molecular clock is present in nearly every cell throughout the body and is organized into a synchronous, hierarchical system by a central clock in the suprachiasmatic nucleus (SCN) that works to temporally control nearly all physiology and behavior (8,9). Notably, disrupted rhythmicity through mutations in the circadian molecular clock genes has been associated with altered reward regulation. In humans, variants in *BMAL1*, *PER*, and *CLOCK* are associated with increased drug use and reward dependence (10–17). In mice, mutations in the *Per*, *Clock*, or *Npas2* genes can significantly alter exploratory drive, cocaine locomotor sensitization, cocaine preference and self-administration/motivation, and alcohol self-administration (18–28). In addition to regulating reward, disruptions to circadian rhythms have also been associated with many other psychiatric disorders often comorbid with substance use (e.g., anxiety, depression, bipolar) (29–32). While together these studies

highlight the role that a functional molecular clock plays in regulating reward-related behaviors, most research to date has primarily focused on molecular clock function in neurons specifically or in whole reward regions, such as the nucleus accumbens (NAc) (33,34). A growing body of work suggests that astrocytes play a critical role in both the regulation of circadian rhythms and reward processing (35,36).

Astrocytes are a highly abundant glial cell type essential for many modulatory and support functions across the central nervous system (37), including regulation of glutamate levels and neurometabolic homeostasis (38–41). Similar to neurons, astrocytes also contain a circadian molecular clock (42–44), and core astrocyte functions are regulated by clock genes (45–47). Several recent studies have demonstrated that astrocyte rhythmicity is also important for regulating circadian rhythmicity. SCN astrocytes show antiphasic rhythmicity relative to SCN neurons, whereby they suppress neuronal activity during the night via regulation of extracellular glutamate (48). This antiphasic relationship is important in regulating SCN circadian timekeeping at both the molecular and behavioral levels (48–50). In addition to regulating circadian rhythms (48), extensive work has shown astrocytic regulation of glutamate in the NAc to be important for reward regulation and drug addiction vulnerability (34,35,51–53). However, while we know that astrocytes regulate circadian rhythms and reward through their roles in the SCN and NAc, respectively, no studies to date have investigated NAc astrocyte rhythmicity in the NAc and/or its potential role in regulating NAc function in the context of reward.

## METHODS AND MATERIALS

See the [Supplement](#) for detailed methods and materials.

### Animals

Aldh111-eGFP/Rpl10a mice were used for characterizing astrocyte rhythmicity (54–56), while *Bmal1* floxed (BMFL; *Bmal1*<sup>lox</sup>) mice were used for loss of rhythmicity experiments (57) (The Jackson Laboratory). Both sexes were used throughout. All mice were maintained on a 12-hour light/dark cycle (lights on: 0700, Zeitgeber time (ZT) 0; lights off: 1900, ZT 12) and provided ad libitum food and water access unless otherwise indicated.

### Immunoprecipitation and RNA Extraction From NAc Astrocytes

NAc tissue was collected from Aldh111-eGFP/Rpl10a mice across 6 times of day (ZT 2, 6, 10, 14, 18, and 22; 8 female/10 male/ZT). Immunoprecipitation of polyribosomes was processed using a modified protocol described previously (58,59). Final eluted NAc astrocyte-specific RNA was used for RNA sequencing.

### RNA Sequencing and Analyses

Following quality/integrity assessment and library preparation, total RNA sequencing was performed using the Illumina NextSeq 500 platform (Illumina, Inc.). After preprocessing and filtering of sequencing data, 12,739 genes remained for further analysis. The JTK CYCLE R package was used to detect circadian rhythmicity of NAc astrocyte-specific genes (60,61). Ingenuity Pathway Analysis (Qiagen) (62) and Metascape (<https://metascape.org/>) (63) were used to identify enriched

molecular pathways/processes in both the top enriched and top rhythmic gene lists.

### RNAscope In Situ Hybridization

For qualitative validation of Aldh111-eGFP/Rpl10a mice, the RNAscope in situ hybridization Fluorescent Multiplex Assay (ACD) was used.

### Viral Placement Surgery and Verification

BMFL mice (8 weeks old) were injected with AAV8-GFAP-Cre-GFP or AAV8-GFAP-eGFP viral vectors (UNC Viral Vector Core) in the NAc, as previously published (25). Mice were allowed  $\geq 2$  weeks to recover before behavioral testing or tissue collection. Viral placement, spread, and specificity were verified using immunofluorescence.

### Behavioral Testing

BMFL mice expressing NAc-specific GFAP-Cre or eGFP (enhanced green fluorescent protein) control were run through a panel of behavioral tests assessing reward-related behaviors at ZT 2–6 or ZT 14–18.

### Gene Expression and Metabolic Assays

In whole NAc tissue from BMFL mice expressing NAc-specific GFAP-Cre or eGFP control, reverse transcriptase quantitative polymerase chain reaction was used to measure astrocyte, metabolic, and glutamate-related gene expression (ZT 4–6). Primers are provided in [Table S1](#).

Both L(+)-lactate and glutathione (GSH) levels were also measured using established and validated colorimetric and luminescent assays (64–67) at ZT 5 and ZT 17.

### Electrophysiology Recordings

Whole-cell patch-clamp recordings were conducted at ZT 4–6 and ZT 16–18 using BMFL NAc-containing sections expressing GFAP-Cre or eGFP control.

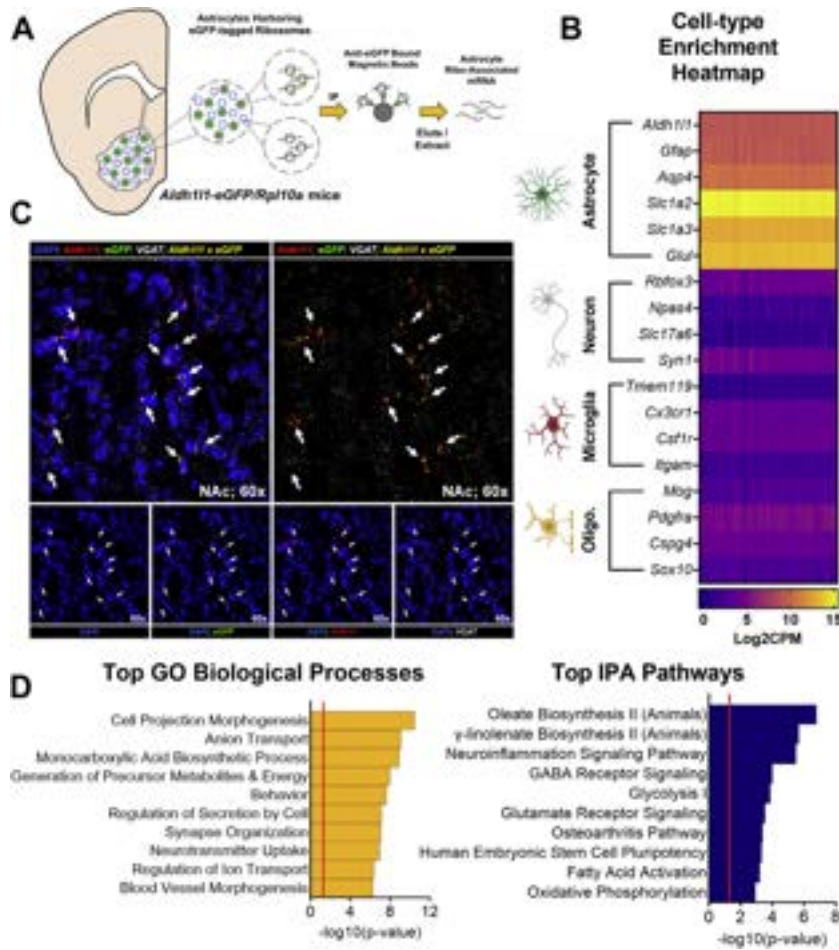
### Statistical Analyses

GraphPad Prism 9 (GraphPad Software) was used for all statistical data analyses. All analyses tested for sex as a variable; if a main effect of sex was detected, sexes are displayed separately. Unless otherwise noted, all data are shown as mean  $\pm$  SEM with  $\alpha = 0.05$ .

## RESULTS

### The NAc Astrocyte Transcriptome Is Highly Rhythmic

Our laboratory and others have previously demonstrated whole NAc rhythmicity at both the cellular and molecular levels (68,69); however, no studies to date have investigated NAc astrocyte rhythmicity. To do this, we used the Aldh111-eGFP/Rpl10a mouse model (70–72), wherein astrocytes exclusively express an eGFP tag on the Rpl10a ribosomal subunit that allows for immunoprecipitation and isolation of astrocyte-specific ribosome-associated messenger RNA (mRNA) ([Figure 1A](#)). We first isolated astrocyte-specific mRNA from whole NAc tissue harvested across 6 times of day in male and female mice following a previously published protocol (58,59). Isolated mRNA was



**Figure 1.** Aldh111-eGFP/Rpl10a mice allow for isolation of astrocyte-specific ribo-associated mRNA. **(A)** Schematic illustrating IP of astrocyte-specific ribo-associated mRNA from whole NAc tissue of Aldh111-eGFP/Rpl10a mice that express an eGFP tag on the ribosomal subunit Rpl10a. **(B)** Isolated mRNA is highly specific for astrocytes, as revealed by a cell-type enrichment heatmap generated from plotting Log2CPM values of established cell-type markers from our RNA sequencing dataset (alternative names: *Slc1a2* = *EAT2* or *Glt-1*; *Slc1a3* = *EAT1* or *Glast*; *Glu1* = *GS*; *Rbfox3* = *NeuN*; *Slc17a6* = *Vglut2*). Columns are the individual samples, and rows are individual genes. Purple indicates low expression, while yellow indicates peak expression. **(C)** Moreover, the fluorophore eGFP is expressed specifically in cells expressing the pan-astrocyte marker *Aldh111*, but not in cells expressing the neuronal GABA transporter VGAT, as determined by RNAscope; arrows indicate cells with co-expression of *Aldh111* and eGFP. **(D)** Following RNA-seq, IPA (62) and Metascape analysis (63) revealed the top canonical pathways and biological processes enriched among the top 200 expressed genes in NAc astrocytes include key metabolic and synaptic neurotransmission functions generally attributed to astrocytes. Notable NAc astrocyte-enriched processes include glycolysis and lactate synthesis, oxidative phosphorylation, glutamate receptor signaling, neurotransmitter uptake (e.g., glutamate), and neuroinflammation signaling. Red line in bar graphs indicates significance threshold of  $p < .05$  or  $-\log_{10}(p \text{ value}) > 1.3$ . eGFP, enhanced green fluorescent protein; GABA, gamma-aminobutyric acid; GO, Gene Ontology; IP, immunoprecipitation; IPA, Ingenuity Pathway Analysis; mRNA, messenger RNA; NAc, nucleus accumbens; RNA-seq, RNA sequencing.

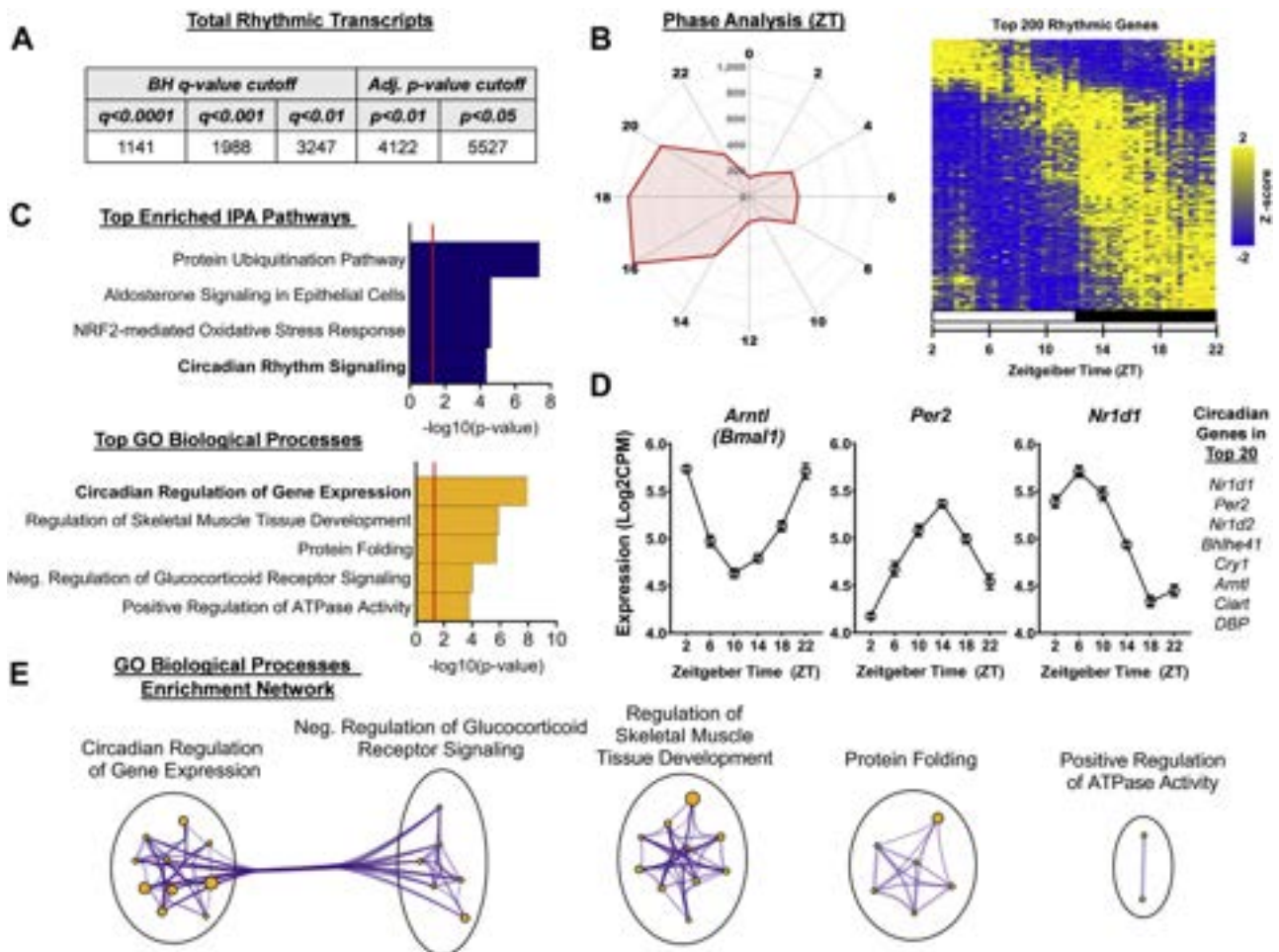
then processed for RNA sequencing analysis, resulting in the detection of 12,739 expressed genes. Notably, cell-type enrichment heatmapping using established cell-type markers (54,56,73–75) revealed a high degree of specificity for astrocyte enrichment (Figure 1B). Moreover, the eGFP tag is highly colocalized with *Aldh111*, a panastrocyte marker (54,55), and not with VGAT, a GABA (gamma-aminobutyric acid) transporter expressed in NAc medium spiny neurons (MSNs) (Figure 1C). Ingenuity Pathway Analysis (62) and Metascape analysis (63) revealed historically astrocyte-associated biological processes and canonical pathways to be enriched among the top 200 expressed genes (Figure 1D), further validating this method and underscoring their potential importance in the NAc. These processes were also highly interconnected, as revealed by an enrichment network analysis (Figure S1).

We next used JTK Cycle, a nonparametric statistical algorithm, to identify transcripts with diurnal variation in expression (60). Of the 12,739 genes we identified, approximately 43.3% display a significant diurnal rhythmicity (Figure 2A). Rank-rank hypergeometric overlap revealed a nearly identical overlap between males and females (Figure S2); thus, analyses proceeded with sexes grouped together. Through assessing

circadian acrophase (time of peak expression), we see the majority of rhythmic genes peak between ZT 14 and ZT 22, coinciding with the mouse’s active phase (Figure 2B). Moreover, pathway enrichment analyses of top rhythmic transcripts revealed circadian pathways/processes among the most enriched (Figure 2C; Figure S3). Also, many of the top 20 rhythmic transcripts are known core clock genes, and their expression patterns align with established expression relationships (Figure 2D). Alongside circadian pathways and processes, important metabolic-relevant functions were also enriched among the top rhythmic genes (Figure 2C). Finally, in an enrichment network analysis of the top rhythmic biological processes, the circadian regulation and glucocorticoid receptor signaling node clusters show a high degree of interconnectivity (Figure 2E; Figure S3).

**Loss of BMAL1 Function in NAc Astrocytes Increases Locomotor Response to Novelty During the Day**

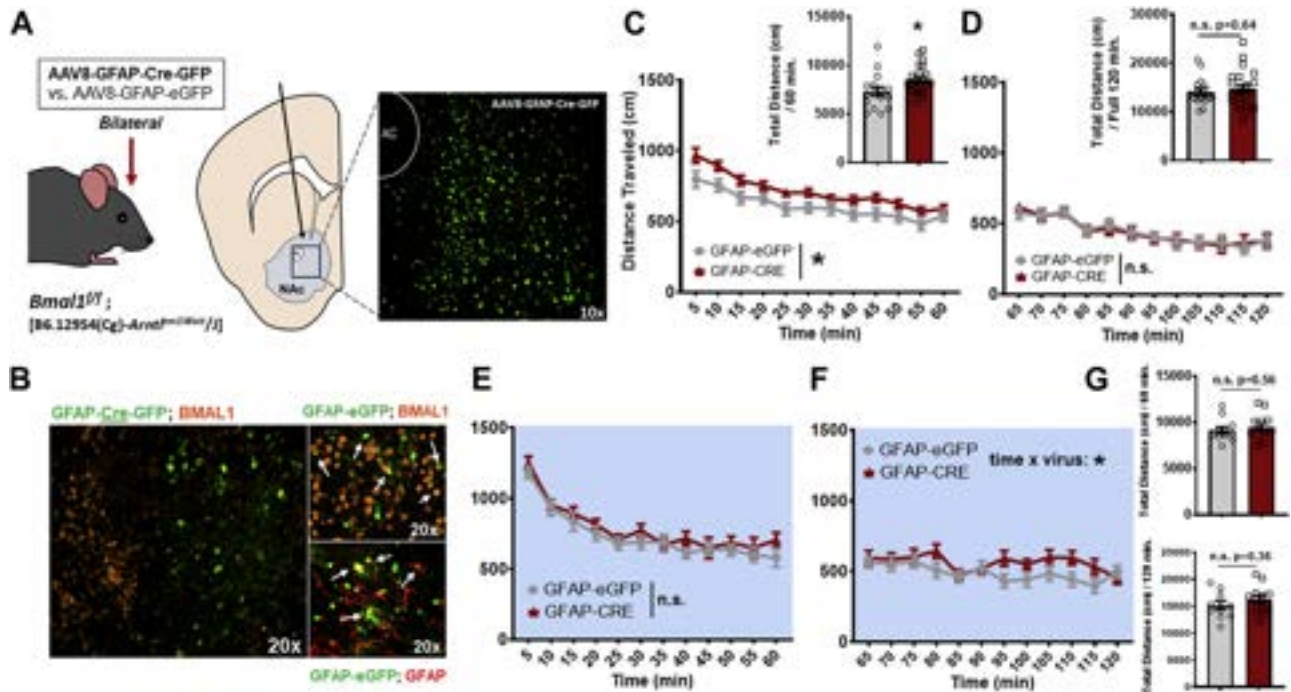
To investigate the functional importance of NAc astrocyte rhythmicity for reward, *Bmal1* floxed mutant mice (*Bmal1<sup>fl/fl</sup>*)



**Figure 2.** NAc astrocytes are highly rhythmic at the transcriptome level. **(A)** Among the 12,739 genes identified in NAc astrocytes, approximately 43.3% of genes exhibited rhythmic expression across the six ZTs sampled (ZT 2, 6, 10, 14, 18, 22;  $n = 4\text{--}5/\text{ZT}$ , JTK\_Cycle), or 5527 genes at the Bonferroni-adjusted  $p$  value cutoff of  $p < .05$ . Even using the most stringent statistical cutoff (BH  $q$ -value cutoff of  $q < 0.0001$ ), approximately 9% of transcripts still display diurnal rhythmicity, or 1141 genes. **(B)** Phase analysis through radar plotting of top rhythmic transcripts ( $p < .05$ ) and heatmap visualization of the top 200 rhythmic genes. The radar plot rhythmic axis represents ZT, while the inner axis is the number of genes with peak expression at a given ZT. Heatmap displays  $z$ -transformed expression for each of the top 200 statistically rhythmic genes (as determined by JTK\_Cycle), ranked in descending order along the  $y$ -axis (rows) and each subject ordered by ZT along the  $x$ -axis (columns). The white bar indicates lights on (ZT 2, 6, 10) and the black bars indicate lights off (ZT 14, 18, 22). **(C)** IPA and Metascape GO Biological Process analysis revealed both circadian and metabolic-related processes enriched among the top rhythmic genes. Notable pathways/processes include circadian rhythm signaling, circadian regulation of gene expression, NRF2-mediated oxidative stress response, regulation of ATPase activity, and glucocorticoid receptor signaling. Red line in bar graphs indicates significance threshold of  $p < .05$  or  $-\log_{10}(p \text{ value}) > 1.3$ . **(D)** Core circadian genes are among the top rhythmic genes and show expression patterns that align with previously established functional relationships (i.e., *Arntl* [gene that encodes BMAL1] is antiphasic with *Per2* and *Nr1d1*). **(E)** Among the top enriched rhythmic GO biological processes, the circadian regulation of gene expression and glucocorticoid receptor signaling node clusters show a high degree of interconnectivity. BH, Benjamini-Hochberg; GO, Gene Ontology; IPA, Ingenuity Pathway Analysis; NAc, nucleus accumbens; ZT, Zeitgeber time.

were stereotaxically injected in the NAc with an AAV (adeno-associated virus) expressing either Cre recombinase or eGFP control under the *Gfap* astrocyte-specific promoter (Figure 3A). NAc expression of GFAP-Cre results in loss of astrocyte molecular clock function and rhythmicity due to deletion of BMAL1's functional domain (49,57,76), which immunofluorescence revealed to be both localized and specific (Figure 3A, B). To assess reward-related behavior, mice were first run through the locomotor response to novelty (LRN) task (ZT 4–6 and ZT 16–18), a behavior established as a predictor of drug-seeking

propensity (77–79). GFAP-Cre mice show a significantly higher LRN during the day, relative to GFAP-eGFP control mice (Figure 3C), measured as total locomotor activity in the first hour of the 2-hour task (Figure 3C and inset). Notably, this increased LRN was not due to any differences in habituation or total activity (Figure 3D and inset). Moreover, no significant differences were observed during the night (Figure 3E–G). However, looking across phase, eGFP mice displayed a significant diurnal variation in LRN that is disrupted in GFAP-Cre mice, driven by increased LRN during the day (Figure S4).



**Figure 3.** Loss of BMAL1 function in NAc astrocytes increases locomotor response to novelty in mice during the day. **(A)** Schematic illustrating the bilateral stereotaxic injection of either AAV8-GFAP-Cre-GFP or GFAP-eGFP virus into the NAc of *Bmal1* floxed mutant mice; injection at AP +1.5, ML ± 1.5, and DV -4.4 μm resulted in a region-specific expression of the virus in the NAc. **(B)** GFAP-Cre-GFP virus resulted in a NAc-specific loss of BMAL1 detection by IF, specifically in GFAP+ cells (i.e., astrocytes). Arrows indicate cells with co-expression. **(C)** In the LRN task, mice with a loss of BMAL1 function in NAc astrocytes (GFAP-Cre) show a significantly greater LRN relative to control mice during the day (virus:  $F_{1,36} = 5.68, *p = .02$ ; time:  $F_{11,396} = 40.41, p < .0001$ ; time × virus:  $F_{11,396} = 0.92, p = .51$ ; inset,  $t_{35} = 2.32, *p = .02$ ). **(D)** This increase in LRN is not driven by differences in habituation or overall locomotor activity across the full task (inset) (virus:  $F_{1,36} = 0.0003, n.s. p = .98$ ; time:  $F_{11,396} = 16.66, p < .0001$ ; time × virus:  $F_{11,396} = 0.26, p = .99$ ; inset,  $t_{35} = 0.46, p = .64$ ). **(E-G)** However, during the night, mice show no significant differences in **(E)** LRN (virus:  $F_{1,22} = 1.017, p = .32$ ; time:  $F_{11,242} = 44.68, p < .0001$ ; time × virus:  $F_{11,242} = 0.62, p = .8$ ), **(F)** habituation (virus:  $F_{1,22} = 2.19, p = .15$ ; time:  $F_{11,242} = 3.28, p = .0003$ ; time × virus:  $F_{11,242} = 1.91, p = .03$ ), or **(G)** overall locomotor differences (top:  $t_{21} = 0.58, p = .56$ ; bottom:  $t_{21} = 0.91, p = .36$ ). White background indicates behavior run during the day (ZT 2–6). Blue background indicates behavior run during the night (ZT 14–18). Mean ± SEM;  $n = 12$ –22; \* $p < .05$ . AP, anterior-posterior; DV, dorsal-ventral; IF, immunofluorescence; LRN, locomotor response to novelty; ML, medial-lateral; NAc, nucleus accumbens; n.s., not significant; ZT, Zeitgeber time.

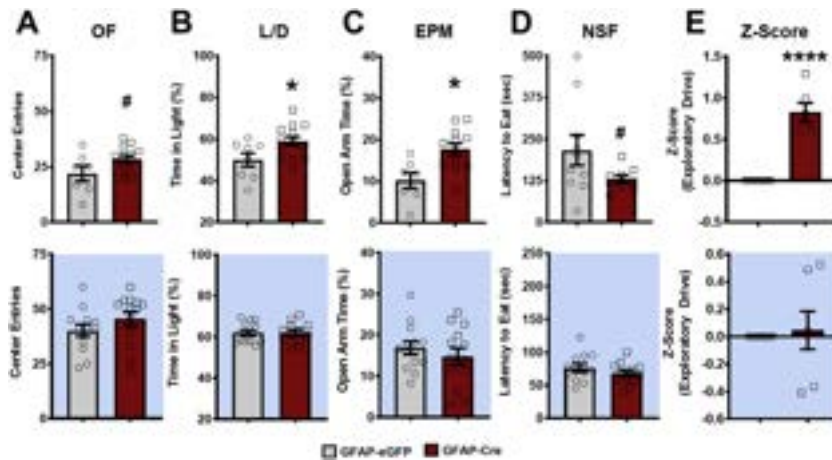
### Loss of BMAL1 Function in NAc Astrocytes Increases Exploratory Drive Behavior During the Day

Alongside LRN, increased exploratory drive and novelty-seeking behavior are also established predictors of vulnerability for drug-seeking and addiction-like behaviors in rodent models (77,78,80–83). Given this association, mice were then run through a battery of exploratory drive/anxiety-related behavioral tasks, including open field, light-dark box test, elevated plus maze, and novelty suppressed feeding, either during the day or at night. Across all four tasks, mice with disrupted NAc astrocyte molecular clock function exhibit significantly elevated exploratory drive during the day, relative to control mice (Figure 4A–D). Following z-normalization of the four behaviors, a method previously shown to increase sensitivity, reliability, and robustness of behavioral phenotyping (84), exploratory drive was found to be significantly greater in GFAP-Cre mice during the day (Figure 4E, top) but not at night (bottom), further underscoring the phenotype. Looking across phase, eGFP mice display a significant diurnal variation in exploratory drive that is disrupted or attenuated in GFAP-Cre mice, driven by effects during the day (Figure S5). Finally, this

phenotype is not attributed to locomotor differences across tasks or motivation to eat in novelty suppressed feeding (Figure S6).

### Loss of BMAL1 Function in NAc Astrocytes Increases Food Self-administration and Motivation During the Day

While LRN and exploratory drive have been established as reward-relevant behaviors, we next investigated reward specifically. Mice were trained either during the day or at night in an operant food self-administration task in which mice discriminate between two levers (active vs. inactive) to receive a food pellet. Mice were first trained on an FR1 schedule, whereby one active lever press is rewarded with one food pellet and inactive lever presses result in no pellets (Figure 5A). On the FR1 schedule, all mice successfully acquired the task (criteria: ≥25 pellets for three sessions) both during the day (Figure 5B) and at night (Figure 5D). While no significant differences were detected between virus groups across phase (Figure 5B, D), a main effect of sex was detected in that male mice exhibited a higher response rate than female mice, both during the day (Figure 5B) and at



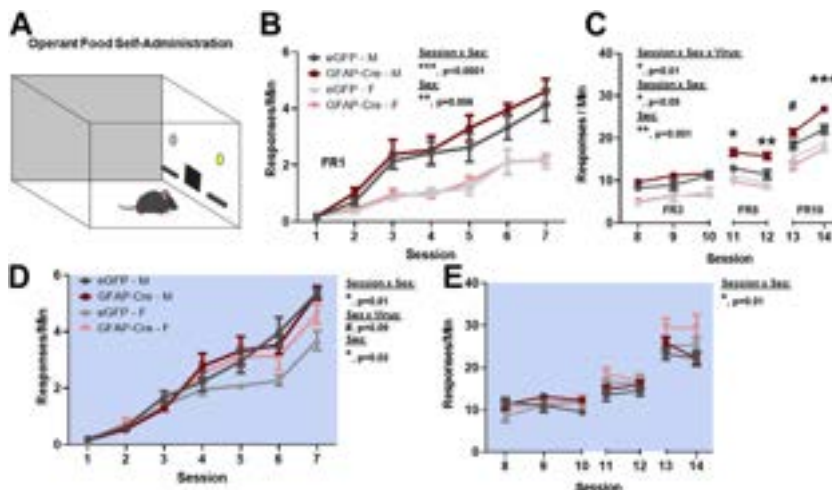
**Figure 4.** Loss of BMAL1 function in NAc astrocytes increases exploratory drive in mice during the day. Both BMFL mice expressing NAc-specific GFAP-Cre or eGFP control were run through a panel of behavioral assays testing exploratory drive.

(A) In the OF task, GFAP-Cre mice show an increased number of center entries relative to eGFP control mice during the day (top:  $t_{18} = 1.91$ ;  $\#p = .07$ ) but not during the night (bottom:  $t_{24} = 1.21$ ;  $p = .24$ ). (B) In the L/D box test, GFAP-Cre mice spend a significantly greater percentage of time in the brightly lit chamber of the arena during the day (top:  $t_{19} = 2.37$ ;  $*p = .02$ ) but not during the night (bottom:  $t_{23} = 0.09$ ;  $p = .92$ ). (C) GFAP-Cre mice also spent significantly more time in the open arms of the EPM during the day (top:  $t_{18} = 2.35$ ;  $*p = .03$ ) but not during the night (bottom:  $t_{24} = 0.81$ ;  $p = .43$ ). (D) Following an overnight food restriction, GFAP-Cre mice had a shorter latency to eat food in the NSF task during the day (top:  $t_{22} = 1.9$ ;  $\#p = .06$ ) but not

during the night (bottom:  $t_{23} = 1.15$ ;  $p = .26$ ). (E) Across the behavioral panel, z-normalization of the four behavioral tasks revealed that a loss of BMAL1 function in NAc astrocytes significantly increases exploratory drive behavior relative to control mice during the day (top:  $t_{11} = 8.14$ ,  $****p < .0001$ ) but not during the night (bottom:  $t_{12} = 0.34$ ;  $p = .74$ ). White background indicates behavior run during the day (ZT 2–6). Blue background indicates behavior run during the night (ZT 14–18). Mean  $\pm$  SEM;  $n = 7$ –13;  $\#p \leq .07$ ,  $*p < .05$ ,  $****p < .0001$ . BMFL, *Bmal1* floxed; eGFP, enhanced green fluorescent protein; EPM, elevated plus maze; L/D, light/dark; NAc, nucleus accumbens; NSF, novelty suppressed feeding; OF, open field; ZT, Zeitgeber time.

night (Figure 5D); this finding is consistent with previous literature showing sex-related differences in operant appetitive learning (85–87). Moreover, looking across phase, while male mice did not show diurnal variation in responding, female eGFP and GFAP-Cre mice both show a significant diurnal variation in food self-administration at FR1 that was enhanced in the GFAP-Cre group (Figure S7). Following FR1 training, food self-administration motivation was tested through the escalation of progressively more demanding FR

schedules. Across progressively harder schedules (FR3, FR5, and FR10), male GFAP-Cre mice (but not females) demonstrate a robust increase in daytime food self-administration, despite the increasingly more demanding schedules (Figure 5C; Figure S8A), but not at night (Figure 5E; Figure S8B). Finally, much like at FR1, female eGFP and GFAP-Cre mice, but not males, show a highly significant diurnal variation in food self-administration motivation (Figure S7).



**Figure 5.** Loss of BMAL1 function in NAc astrocytes increases operant food self-administration and motivation during the day. To assess natural reward and reward motivation, both BMFL mice expressing NAc-specific GFAP-Cre or eGFP control were trained to self-administer food pellets in an operant food self-administration task both during the day and at night. (A) Mice were trained to discriminate between an active and an inactive lever, whereby active lever pressing resulted in an FR of food pellets. (B) On an FR1 schedule (i.e., 1 lever press = 1 pellet), GFAP-Cre mice successfully learn to self-administer food (main effect of session:  $F_{6,60} = 59.63$ ,  $p < .0001$ ) but show no significant differences relative to control mice (virus:  $F_{1,10} = 1.68$ ,  $p = .22$ ). In both virus groups, males show a significantly greater response rate relative to females (sex:  $F_{0,4,4} = 37.11$ ,  $**p = .006$ ; session  $\times$  sex:  $F_{3,5,35,2} = 8.52$ ,  $***p < .0001$ ). (C) To test reward motivation, mice were tested across increasingly difficult FR schedules (i.e., FR3, 3 presses = 1 pellet; FR5, 5

presses = 1 pellet; FR10, 10 presses = 1 pellet). GFAP-Cre male mice not only show a robust maintenance but also a significant increase in food self-administration rate across FR schedules during the day relative to control counterparts (session  $\times$  sex  $\times$  virus:  $F_{6,59} = 2.96$ ,  $*p = .01$ ; sex  $\times$  virus:  $F_{1,10} = 5.09$ ,  $*p < .05$ ; sex:  $F_{0,6,6} = 34.92$ ,  $**p = .001$ ). No effect of the virus was seen in female mice. (D) During the night, GFAP-Cre mice also successfully learn to self-administer food (main effect of session:  $F_{7,70} = 164.4$ ,  $p < .0001$ ), with a main effect of sex (sex:  $F_{0,37,3,7} = 15.01$ ,  $*p = .03$ ; session  $\times$  sex:  $F_{2,8,25,96} = 3.55$ ,  $*p = .03$ ; sex  $\times$  virus:  $F_{1,10} = 5.05$ ,  $*p = .04$ ), but no significant effects of virus (virus:  $F_{1,10} = 2.29$ ,  $p = .16$ ). (E) During the night, no significant differences are seen in motivation as mice are tested across increasing FR schedules (session  $\times$  sex  $\times$  virus:  $F_{6,58} = 1.1$ ,  $p = .37$ ; sex  $\times$  virus:  $F_{1,10} = 0.09$ ,  $p = .76$ ; sex:  $F_{0,37,3,67} = 2.17$ ,  $p = .17$ ; session  $\times$  sex:  $F_{2,5,24,32} = 4.75$ ,  $*p = .01$ ). White background indicates behavior run during the day (ZT 2–6). Blue background indicates behavior run during the night (ZT 14–18). Mean  $\pm$  SEM;  $n = 6$ ;  $\#p = .07$ ,  $*p < .05$ ,  $**p < .01$ ,  $***p = .0001$ . BMFL, *Bmal1* floxed; eGFP, enhanced green fluorescent protein; F, female; FR, fixed ratio; M, male; NAc, nucleus accumbens; ZT, Zeitgeber time.

### Loss of BMAL1 Function in NAc Astrocytes Disrupts Metabolic Homeostasis

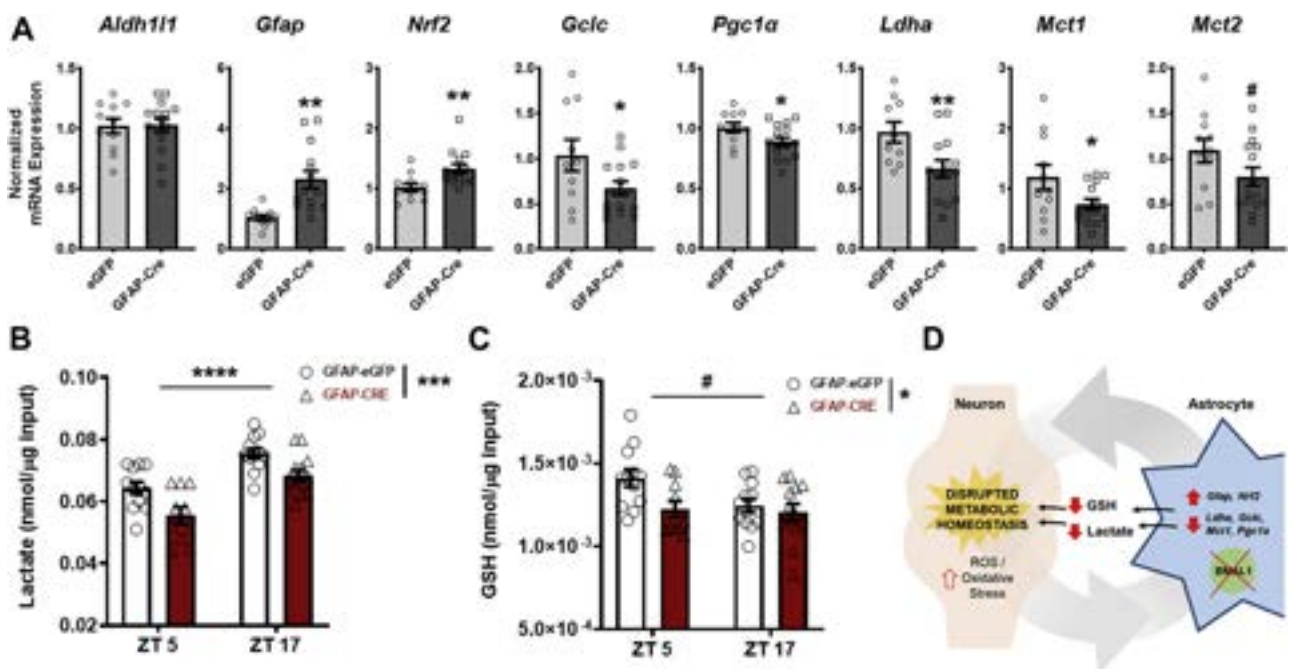
Given that astrocytes play a crucial role in regulating cellular metabolic state, we next wanted to investigate how disrupting NAc astrocyte molecular clock function affects this key function. Using reverse transcriptase quantitative polymerase chain reaction in GFAP-Cre or eGFP whole NAc tissue, we first assessed expression of key astrocyte and metabolic state relevant genes during the day (ZT 4–6; i.e., time of behavioral phenotype) (Figure 6A). No significant difference was seen in *Aldh11* expression. However, GFAP-Cre mice exhibit a significant upregulation of *Gfap* and *Nrf2*, established markers of astrocyte reactivity and oxidative stress-induced antioxidant response, respectively (88,89). Moreover, GFAP-Cre mice exhibited a significant downregulation of *Gclc*, *Pgc1 $\alpha$* , *Ldha*, *Mct1*, and *Mct2*, all genes relevant for GSH production, mitochondrial function, and lactate synthesis/shuttling, respectively.

We next wanted to determine the concentration of lactate and GSH in the NAc following disruption of NAc astrocyte molecular clock function. Astrocytes synthesize and shuttle both lactate and GSH to neurons to help support their

metabolic demands and prevent oxidative stress, respectively (40,41,90). To measure lactate, NAc tissue from GFAP-Cre mice was run through an L(+)-lactate colorimetric enzyme-linked immunosorbent assay. Alongside the reductions in lactate-related gene expression (Figure 6A), GFAP-Cre mice exhibited a significant reduction in lactate concentration across time of day relative to control mice (Figure 6B). NAc lactate concentration exhibits diurnal variation in eGFP control mice, which was disrupted in GFAP-Cre mice. Alongside lactate measures, NAc tissue from GFAP-Cre mice was also run through a GSH luminescent enzyme-linked immunosorbent assay. Much like lactate levels, GFAP-Cre mice exhibit significantly reduced GSH concentration across time of day relative to control mice (Figure 6C). Notably, control mice also exhibit diurnal variation in NAc GSH concentration that is significantly reduced in GFAP-Cre mice.

### Loss of BMAL1 Function in NAc Astrocytes Alters Excitatory Synaptic Transmission Onto Neighboring MSNs

To investigate the impact that astrocyte molecular clock disruption may have on NAc synaptic transmission, we

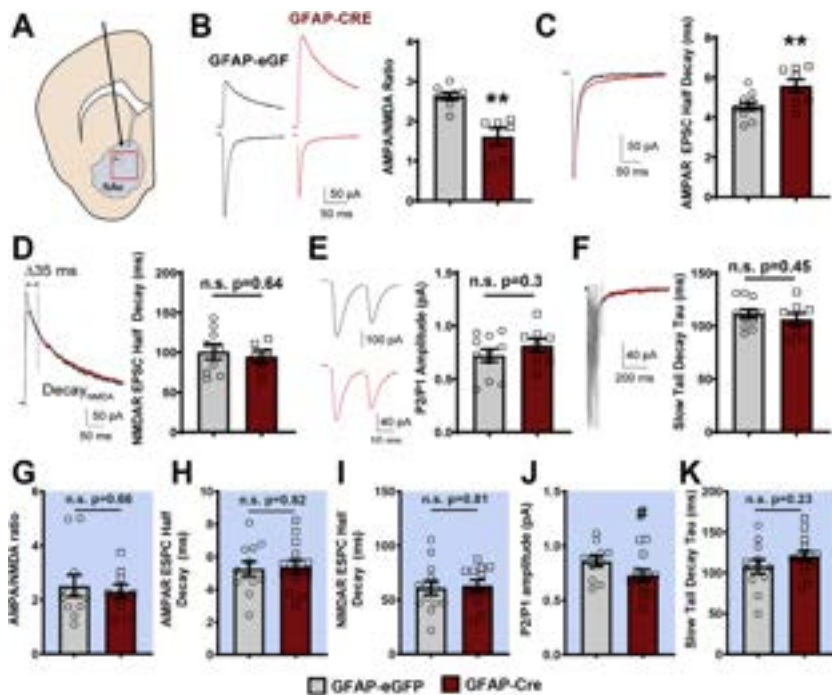


**Figure 6.** Loss of BMAL1 function in NAc astrocytes disrupts NAc metabolic homeostasis. NAc tissue from both BMFL mice expressing NAc-specific GFAP-Cre or eGFP control was processed through a range of molecular assays assessing factors relevant for regulation of cellular metabolic state. **(A)** Through RT-qPCR, a panel of metabolic genes was assessed in the whole NAc of GFAP-Cre and eGFP control mice (ZT 4–6). While there were no differences in the astrocyte marker *Aldh11* ( $t_{24} = 0.09$ ,  $p = .92$ ), GFAP-Cre mice showed significantly elevated expression of *Gfap* ( $t_{23} = 3.74$ ,  $**p = .001$ ) and *Nrf2* ( $t_{23} = 2.87$ ,  $**p = .008$ ), markers of astrocyte activation and oxidative stress antioxidant response. GFAP-Cre mice also showed significant reductions in genes relevant for GSH production (*Gclc*;  $t_{23} = 2.15$ ,  $*p < .05$ ), mitochondrial biogenesis (*Pgc1 $\alpha$* ;  $t_{24} = 2.13$ ,  $*p < .05$ ), and lactate synthesis and shuttling (*Ldha*,  $t_{22} = 2.75$ ,  $*p = .01$ ; *Mct1*,  $t_{24} = 2.18$ ,  $*p < .05$ ; *Mct2*,  $t_{24} = 1.77$ ,  $#p = .08$ ). **(B, C)** Moreover, loss of BMAL1 function in NAc astrocytes significantly reduces the concentration of **(B)** lactate (time:  $F_{1,43} = 35.76$ ,  $****p < .0001$ ; virus:  $F_{1,43} = 15.79$ ,  $***p = .0003$ ; interaction:  $F_{1,43} = 0.20$ ,  $p = .65$ ) and **(C)** GSH (time:  $F_{1,43} = 3.56$ ,  $#p = .06$ ; virus:  $F_{1,43} = 5.31$ ,  $*p = .02$ ; interaction:  $F_{1,43} = 2.36$ ,  $p = .13$ ) in the whole NAc across time of day. **(D)** The effects on key metabolic genes, lactate, and GSH are hypothesized to disrupt neurometabolic homeostasis, potentially leading to downstream effects on NAc-regulated behavior. Gene expression normalized to 18s. Mean  $\pm$  SEM.  $n = 10$ –15.  $#p = .06$ ;  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$ ,  $****p < .0001$ . eGFP, enhanced green fluorescent protein; GSH, glutathione; NAc, nucleus accumbens; ROS, reactive oxygen species; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; ZT, Zeitgeber time.

performed whole-cell recordings from the principal MSNs in the NAc from both GFAP-Cre and eGFP control mice during the day and night (Figure 7A). Following the disruption of NAc astrocyte molecular clock function, the evoked AMPA/NMDA excitatory postsynaptic current (EPSC) ratio was significantly decreased in GFAP-Cre mice during the day (Figure 7B), which was accompanied by a daytime increase in AMPA (but not NMDA) receptor EPSC decay kinetics (Figure 7C, D), i.e., suggesting postsynaptic glutamate receptor changes, particularly that of AMPA receptors. Furthermore, the evoked EPSCs exhibited similar paired-pulse ratios across phases (Figure 7E), suggesting that the presynaptic release probabilities of mixed glutamatergic inputs were grossly unaltered under basal transmission. Following a train of stimuli, the slow EPSC tail also showed similar decay kinetics across phases (Figure 7F), suggesting comparable glutamate clearance between groups. No significant differences in any of these parameters were detected at night (Figure 7G-I, K; Figure S9), although a trending decrease in paired-pulse ratio was observed (Figure 7J). Alongside these electrophysiological changes, GFAP-Cre mice also show a significant upregulation of glutamate-related gene expression in the whole NAc during the day, with upregulation of nearly all AMPA receptor subunits (Figure 8).

DISCUSSION

In this study, we demonstrate for the first time that astrocytes in the NAc have strong molecular rhythms, and this circadian function is important for overall NAc function. Using RNA sequencing across six times of day, we reveal that approximately 43.3% of detected genes in the NAc astrocyte transcriptome show significant diurnal variation, with circadian and metabolic processes as the top enriched pathways/processes among these rhythmic genes. Furthermore, we demonstrate that mice with genetic disruption of NAc astrocyte molecular clock function have significantly altered reward-related behavior, including phase-dependent increases in exploratory drive, as well as increases in food self-administration and motivation. Expanding on the behavioral phenotype, these mice also have disrupted NAc metabolic homeostasis, as revealed by downregulation of lactate and GSH, as well as altered metabolic-related gene expression. Finally, through electrophysiological whole-cell recordings, we provide evidence that disrupting NAc astrocyte molecular clock function also alters excitatory synaptic transmission onto neighboring MSNs via decreased AMPA receptor signaling alongside upregulated glutamate-related gene expression. Taken together, these novel findings underscore the functional significance of NAc astrocytes and the degree to which their



**Figure 7.** Loss of BMAL1 function in the NAc alters excitatory synaptic transmission onto neighboring MSNs during the day. Slice electrophysiology was used to assess the effects of loss of BMAL1 function in NAc astrocytes on glutamatergic signaling in neighboring MSNs during the light and dark phases. (A) Location of stereotaxic injection of AAV8-GFAP-eGFP or AAV8-GFAP-Cre-GFP viral vectors and region targeted for slice electrophysiology experiments. (B) Representative traces (left) and quantification (right) of the AMPA/NMDA ratio. GFAP-Cre mice had a significantly lower AMPA/NMDA ratio than GFAP-eGFP control mice ( $t_{12} = 4.753$ ,  $**p < .01$ , unpaired  $t$  test). (C) Representative traces (left) and quantification (right) showing that GFAP-Cre mice have slower AMPA decay kinetics, as measured by AMPAR half decay, than GFAP-eGFP control mice ( $t_{17} = 2.903$ ,  $**p < .01$ , unpaired  $t$  test). (D) However, there was no difference in NMDA receptor kinetics between GFAP-Cre mice and control mice (representative traces, left; quantification, right;  $t_{13} = 0.469$ ,  $p = .647$ , unpaired  $t$  test). (E) Both GFAP-Cre and eGFP mice displayed a similar paired-pulse ratio (P2/P1 [i.e., EPSC2/EPSC1], measured at 20 ms ISI;  $t_{17} = 1.063$ ,  $p = .303$ , unpaired  $t$  test; representative traces, left; quantification, right). (F) Moreover, there was no difference in the tau of the slow tail decay after a train of high frequency extracellular stimulation ( $5 \times 0.5$  ms  $50 \mu$ A pulses at 50 Hz) between GFAP-Cre and eGFP mice

(measured from 35 ms following the peak of the last AMPAR EPSC;  $t_{17} = 0.769$ ,  $p = .453$ , unpaired  $t$  test; representative traces, left; quantification, right). Notably, when recording at night, no significant differences were seen in (G) AMPA/NMDA ratio ( $t_{18} = 0.4396$ ,  $p = .665$ , unpaired  $t$  test), (H) AMPA decay kinetics ( $t_{24} = 0.2177$ ,  $p = .829$ , unpaired  $t$  test), (I) NMDA decay kinetics ( $t_{23} = 0.2336$ ,  $p = .817$ , unpaired  $t$  test), (J) paired-pulse ratio ( $t_{22} = 1.781$ ,  $^{\#}p = .088$ , unpaired  $t$  test), although trending, nor (K) tau of the slow tail decay ( $t_{24} = 1.225$ ,  $p = .2323$ , unpaired  $t$  test) between GFAP-Cre and eGFP control mice. Red traces indicate GFAP-Cre group. Recordings performed ZT 4–6 and ZT 16–18. Blue background indicates behavior run during the night (ZT 14–18). Mean  $\pm$  SEM,  $n = 6$ –14.  $**p < .01$ . AMPAR, AMPA receptor; eGFP, enhanced green fluorescent protein; EPSC, excitatory postsynaptic current; ISI, interstimulus interval; MSN, medium spiny neuron; NAc, nucleus accumbens; NMDAR, NMDA receptor; n.s., not significant; ZT, Zeitgeber time.

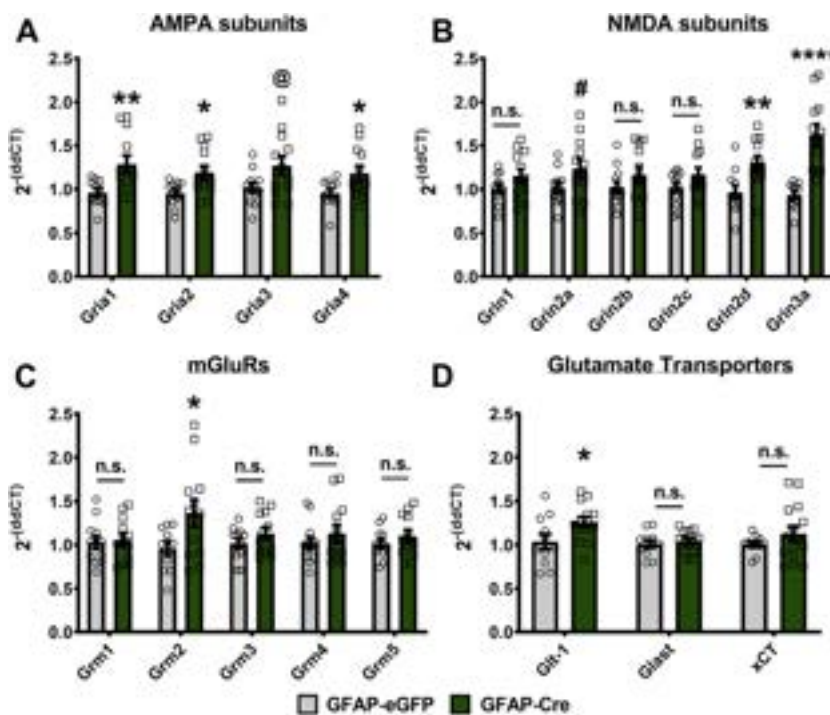


circadian regulation is essential for NAc function and NAc-regulated behaviors.

Of particular interest, a potential mechanism underlying our GFAP-Cre phenotype may be the uncoupling of circadian regulation of neurons from astrocytes. Astrocytes in the SCN play an integral role in regulating neuronal rhythmicity through circadian controlled uptake/release of glutamate and modulation of GABAergic tone and receptor signaling (48,91). Moreover, disrupting circadian astrocyte function in the SCN causes a lengthening of the circadian period (i.e., an inability to keep rhythms consolidated to ~24 hours) in both SCN molecular rhythms and overall behavior rhythms (49). Notably, much like the SCN, the NAc consists primarily of GABAergic neurons, and NAc astrocytes play a crucial role in maintaining glutamate homeostasis (35,92,93). Similar to how SCN astrocytic regulation of extracellular glutamate in a diurnal manner is important for regulating circadian rhythms (48), astrocytic regulation of glutamate in the NAc is thought to contribute to reward regulation and drug addiction vulnerability (34,35,51–53). This is further supported here through pathway analyses revealing glutamate and GABA receptor signaling and neurotransmitter uptake (glutamate) pathways/processes enriched among NAc astrocytes' top 200 expressed genes. Likewise, we find that disrupting NAc astrocyte circadian function alters AMPA receptor signaling on neighboring MSNs by slowing the decay kinetics and

reducing their response relative to that of NMDA receptors, alongside increasing AMPA subunit, specific NMDA subunit, metabotropic glutamate receptor, and glutamate transporter mRNA levels (perhaps compensatory). Previous work in our laboratory has shown that mice with a global functional mutation in CLOCK also have a decreased AMPA/NMDA ratio in NAc MSNs and exhibit some of the same behaviors described here (21,94). While not investigated, it is possible these previously reported *Clock* phenotypes are being driven by a loss of CLOCK function in astrocytes. This can be determined in future studies. Taken together, further investigation is needed into how disrupting NAc astrocyte molecular clock function affects glutamate signaling and/or its effects on neuronal rhythmicity in the context of reward.

In addition to regulating glutamate levels, astrocytes play a central role in the maintenance of neuronal metabolic homeostasis, including synthesis and shuttling of both lactate and GSH (38–41). The astrocyte-to-neuron lactate shuttle hypothesis proposes that increased neuronal activity and subsequent glutamate uptake by astrocytes triggers astrocytic intake of glucose from blood capillaries, increasing glycolysis-mediated production of lactate (a primary central nervous system energy substrate) and increasing shuttling of lactate to neurons, via monocarboxylate transporters, to sustain their energetic needs (95–98). Lactate synthesis and shuttling are known to be regulated by the molecular clock



**Figure 8.** Loss of BMAL1 function in NAc astrocytes upregulates NAc glutamate-related gene expression during the day. NAc tissue from BMFL mice expressing NAc-specific GFAP-Cre or eGFP control was processed through RT-qPCR assessing daytime whole NAc gene expression of AMPA receptor subunits, NMDA receptor subunits, mGluRs, and astrocyte-specific glutamate transporters (ZT 4–6). **(A)** GFAP-Cre mice show significantly upregulated expression of nearly all AMPA receptor subunits, including *Gria1* ( $t_{21} = 2.874$ ,  $**p < .01$ , unpaired *t* test), *Gria2* ( $t_{21} = 2.583$ ,  $*p = .017$ ), *Gria4* ( $t_{21} = 2.267$ ,  $*p < .05$ ), and a trending increase in *Gria3* ( $t_{22} = 2.072$ ,  $^{\circ}p = .05$ ), all relative to eGFP control mice. **(B)** Looking at NMDA receptor subunit gene expression, only *Grin2d* ( $t_{21} = 3.156$ ,  $**p = .0048$ ) and *Grin3a* ( $t_{21} = 5.246$ ,  $****p < .0001$ ) show significant upregulation, with *Grin2a* ( $t_{22} = 1.92$ ,  $^{\#}p = .06$ ) showing a trending increase, relative to control mice. No significant differences between GFAP-Cre and eGFP control were detected for *Grin1* ( $t_{22} = 1.491$ ,  $p = .15$ ), *Grin2b* ( $t_{22} = 1.276$ ,  $p = .21$ ), and *Grin2c* ( $t_{22} = 1.555$ ,  $p = .13$ ), while *Grin3b* was not detectable at all in our whole NAc tissue samples (data not shown). **(C)** In addition to AMPA and NMDA receptor subunits, we also investigated the expression of the G-protein-coupled mGluRs. GFAP-Cre significantly increased *Grm2* relative to eGFP control mice ( $t_{21} = 2.257$ ,  $*p < .05$ ), but no significant differences were detected in *Grm1* ( $t_{22} = 0.2711$ ,  $p = .78$ ), *Grm3* ( $t_{22} = 1.295$ ,  $p = .2$ ), *Grm4*

( $t_{22} = 0.8764$ ,  $p = .39$ ), or *Grm5* ( $t_{22} = 0.9804$ ,  $p = .33$ ) expression in the NAc. **(D)** Finally, expression of astrocyte-specific glutamate transporters was also assessed in the whole NAc of GFAP-Cre and eGFP control mice. *Glt-1* (i.e., *SLC1A2* or *EAAT2*) was significantly upregulated in GFAP-Cre mice relative to controls ( $t_{23} = 2.225$ ,  $*p < .05$ ), while no significant differences were seen with *Glast* (i.e., *SLC1A3* or *EAAT1*;  $t_{23} = 0.5574$ ,  $p = .58$ ) or the *xCT* cystine-glutamate antiporter (i.e., *SLC7A11*;  $t_{24} = 0.2397$ ,  $p = .23$ ) expression. Gene expression normalized to 18s. Mean  $\pm$  SEM,  $n = 11$ –15.  $^{\#}p = .06$ ;  $^{\circ}p = .05$ ;  $*p < .05$ ;  $**p < .01$ ,  $****p < .0001$ . BMFL, *Bmal1* floxed; eGFP, enhanced green fluorescent protein; NAc, nucleus accumbens; mGluR, metabotropic glutamate receptor; n.s., not significant; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; ZT, Zeitgeber time.

and show diurnal variation (99–104). Here, we demonstrate that disrupting NAc astrocyte clock function leads to a significant downregulation of NAc lactate levels across time of day, as well as genes important for lactate synthesis (*Ldha*) and shuttling (*Mct1* and *Mct2*). This is of particular interest for reward regulation given the interconnectivity of circadian rhythms, neuronal metabolism, and drug reward (105). While studies in the amygdala find that blocking lactate shuttling attenuates cocaine reward (106–108), in the NAc, the opposite appears to be true. In a recent study, blocking glucocorticoid-mediated lactate release (i.e., decreased lactate) significantly increased morphine preference that could be normalized by restoring lactate (109). This model aligns with our findings. Not only is there extensive literature suggesting a dynamic interaction between the circadian system and glucocorticoids in the context of reward (110,111), but also here we show that in NAc astrocytes, glucocorticoid receptor signaling is an enriched rhythmic pathway with high interconnectivity with the circadian regulation pathway. This may partly explain our molecular findings, in that glucocorticoid receptor signaling regulates both lactate production and NRF2 pathway activation (109,112,113). However, future investigation is needed into this relationship and how it may underly our model.

Moreover, much like the astrocyte-to-neuron lactate shuttle hypothesis, activation of the NRF2 antioxidant signaling pathway in astrocytes is also coupled with neuronal glutamatergic neurotransmission, whereby glutamate triggers its activation (40,114–116). NRF2 is also activated directly by the presence of reactive oxygen species, inducing expression of GSH enzymes and many other antioxidants (117–119) to remove reactive oxygen species that would otherwise damage the cell, also known as oxidative stress (120). Here, we show monocarboxylic acid biosynthesis (i.e., lactate), glycolysis, oxidative phosphorylation, and generation of precursor metabolites/energy to be enriched among NAc astrocytes' top expressed genes—all processes known to generate reactive oxygen species and potential oxidative stress. Moreover, circadian analysis revealed NRF2-mediated oxidative stress response and ATPase activity to be enriched among the top rhythmic transcripts. Further supporting these enriched processes, disrupting NAc astrocyte molecular clock function significantly downregulates NAc GSH levels as well as related genes, and both *Gfap* and *Nrf2* expression are significantly upregulated, known markers of astrocyte reactivity and oxidative stress-induced antioxidant response. Given the growing association between oxidative stress and SUDs and the therapeutic potential of antioxidants that act on astrocytes (e.g., N-acetylcysteine, a GSH precursor) (121,122), future studies will investigate this relationship as it relates to circadian reward function in the NAc.

Finally, throughout our studies, male mice and female mice were grouped together when no sex differences were statistically detected. Only one sex difference was observed, wherein disrupting NAc astrocyte molecular clock function produced a significant increase in light-phase food self-administration and motivation in male mice. This is particularly intriguing given our recent findings that *Npas2* mutant mice show a phase- and sex-specific increase in cocaine self-administration, where *Npas2* mutation increased dark-phase

self-administration behaviors in female mice (28). Notably, ovariectomy normalizes the phenotype, suggesting circulating hormones to be underlying this sex-specific effect (28). While not investigated here, it may be interesting to explore the role of circulating hormones in the sex effects seen in this study. In addition, these nearly opposing phenotypes may be driven by differential circadian effects of neurons versus astrocytes in the NAc, given that NPAS2 is primarily enriched in D1-MSNs (25,123). Although our rank-rank hypergeometric overlap analysis revealed a nearly identical overlap in NAc astrocyte rhythmic transcripts between sexes, this sequencing analysis was limited to just astrocytes and in nonexperimentally manipulated mice. Future studies will investigate sex-specific transcriptional changes in both astrocytes and neurons that may arise following disruption of NAc astrocyte molecular clock function.

Collectively, the results of this study demonstrate for the first time that NAc astrocytes exhibit a high degree of diurnal rhythmicity at the transcriptome level, with metabolic-relevant pathways/processes enriched among the top rhythmic genes. Moreover, our data reveal that this circadian astrocyte function is important not only for the maintenance of metabolic homeostasis but also for glutamatergic signaling to MSNs and downstream regulation of NAc-regulated behaviors. While this circadian NAc astrocyte function most obviously has implications in the context of reward regulation, as shown here, it also has implications across other psychiatric disorders known to involve the NAc. Ultimately, these data further our understanding of the relationship between circadian rhythms, metabolic state, and reward circuitry, and reveal a fundamental role of astrocyte rhythmicity in regulating NAc function.

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DDB-K and CAM conceived and designed the project. DDB-K and CAM wrote the manuscript with help from JNB and YHH. All authors reviewed and edited the manuscript prior to submission. DDB-K performed the experiments with assistance from KDK, JNB, MAH, LMD, CAV, and RWL. JNB performed electrophysiological recordings and analysis under the guidance of YHH. WZ performed biostatistical analysis of RNA sequencing data under the guidance of GCT. DDB-K analyzed all behavioral and molecular data and created all figures throughout.

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## ARTICLE INFORMATION

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## REFERENCES

- Hedegaard H, Miniño AM, Warner M (2020): Drug overdose deaths in the United States, 1999–2018. *NCHS Data Brief* (356):1–8.
- National Institute on Drug Abuse: Costs of substance abuse. Available at: <https://www.drugabuse.gov/drug-topics/trends-statistics/costs-substance-abuse>. Accessed October 20, 2021.
- National Institute on Drug Abuse: Drugs, brains, and behavior: The science of addiction. Treatment and recovery. Available at: <https://www.drugabuse.gov/publications/drugs-brains-behavior-science-addiction/treatment-recovery>. Accessed October 20, 2021.
- Hasler BP, Smith LJ, Cousins JC, Bootzin RR (2012): Circadian rhythms, sleep, and substance abuse. *Sleep Med Rev* 16:67–81.
- Angarita GA, Emadi N, Hodges S, Morgan PT (2016): Sleep abnormalities associated with alcohol, cannabis, cocaine, and opiate use: A comprehensive review. *Addict Sci Clin Pract* 11:9.
- Logan RW, Williams WP 3rd, McClung CA (2014): Circadian rhythms and addiction: Mechanistic insights and future directions. *Behav Neurosci* 128:387–412.
- Reppert SM, Weaver DR (2002): Coordination of circadian timing in mammals. *Nature* 418:935–941.
- Partch CL, Green CB, Takahashi JS (2014): Molecular architecture of the mammalian circadian clock. *Trends Cell Biol* 24:90–99.
- Takahashi JS (2017): Transcriptional architecture of the mammalian circadian clock. *Nat Rev Genet* 18:164–179.
- Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC, et al. (2005): The clock gene *Per2* influences the glutamatergic system and modulates alcohol consumption [published correction appears in *Nat Med* 2005; 11:233]. *Nat Med* 11:35–242.
- Comasco E, Nordquist N, Göktürk C, Aslund C, Hallman J, Orelund L, Nilsson KW (2010): The clock gene *PER2* and sleep problems: Association with alcohol consumption among Swedish adolescents. *Ups J Med Sci* 115:41–48.
- Kovanen L, Saarikoski ST, Haukka J, Pirkola S, Aromaa A, Lönnqvist J, Partonen T (2010): Circadian clock gene polymorphisms in alcohol use disorders and alcohol consumption. *Alcohol Alcohol* 45:303–311.
- Sjöholm LK, Kovanen L, Saarikoski ST, Schalling M, Lavebratt C, Partonen T (2010): *CLOCK* is suggested to associate with comorbid alcohol use and depressive disorders. *J Circadian Rhythms* 8:1.
- Dong L, Bilbao A, Laucht M, Henriksson R, Yakovleva T, Ridinger M, et al. (2011): Effects of the circadian rhythm gene *period 1* (*per1*) on psychosocial stress-induced alcohol drinking. *Am J Psychiatry* 168:1090–1098.
- Shumay E, Fowler JS, Wang GJ, Logan J, Alia-Klein N, Goldstein RZ, et al. (2012): Repeat variation in the human *PER2* gene as a new genetic marker associated with cocaine addiction and brain dopamine D2 receptor availability. *Transl Psychiatry* 2:e86.
- Tsuchimine S, Yasui-Furukori N, Kaneda A, Kaneko S (2013): The *CLOCK* C3111T polymorphism is associated with reward dependence in healthy Japanese subjects. *Neuropsychobiology* 67:1–5.
- Bi J, Gelernter J, Sun J, Kranzler HR (2014): Comparing the utility of homogeneous subtypes of cocaine use and related behaviors with DSM-IV cocaine dependence as traits for genetic association analysis. *Am J Med Genet B Neuropsychiatr Genet* 165B:148–156.
- Abarca C, Albrecht U, Spanagel R (2002): Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proc Natl Acad Sci U S A* 99:9026–9030.
- McClung CA, Sidiropoulou K, Vitaterna M, Takahashi JS, White FJ, Cooper DC, Nestler EJ (2005): Regulation of dopaminergic transmission and cocaine reward by the *Clock* gene. *Proc Natl Acad Sci U S A* 102:9377–9381.
- Becker-Krail DD, Parekh PK, Ketchesin KD, Yamaguchi S, Yoshino J, Hildebrand MA, et al. (2022): Circadian transcription factor NPAS2 and the NAD<sup>+</sup>-dependent deacetylase SIRT1 interact in the mouse nucleus accumbens and regulate reward. *Eur J Neurosci* 55:675–693.
- Roybal K, Theobald D, Graham A, DiNieri JA, Russo SJ, Krishnan V, et al. (2007): Mania-like behavior induced by disruption of *CLOCK*. *Proc Natl Acad Sci U S A* 104:6406–6411.
- Ozburn AR, Larson EB, Self DW, McClung CA (2012): Cocaine self-administration behaviors in *Clock* $\Delta$ 19 mice. *Psychopharmacology (Berl)* 223:169–177.
- Ozburn AR, Falcon E, Mukherjee S, Gillman A, Arey R, Spencer S, McClung CA (2013): The role of clock in ethanol-related behaviors. *Neuropsychopharmacology* 38:2393–2400.
- Turner JR, Ecker LE, Briand LA, Haydon PG, Blendy JA (2013): Cocaine-related behaviors in mice with deficient gliotransmission. *Psychopharmacology (Berl)* 226:167–176.
- Ozburn AR, Falcon E, Twaddle A, Nugent AL, Gillman AG, Spencer SM, et al. (2015): Direct regulation of diurnal *Drd3* expression and cocaine reward by NPAS2. *Biol Psychiatry* 77:425–433.
- Ozburn AR, Kern J, Parekh PK, Logan RW, Liu Z, Falcon E, et al. (2017): NPAS2 regulation of anxiety-like behavior and GABA<sub>A</sub> receptors. *Front Mol Neurosci* 10:360.
- Parekh PK, Logan RW, Ketchesin KD, Becker-Krail D, Shelton MA, Hildebrand MA, et al. (2019): Cell-type-specific regulation of nucleus accumbens synaptic plasticity and cocaine reward sensitivity by the circadian protein, NPAS2. *J Neurosci* 39:4657–4667.
- DePoy LM, Becker-Krail DD, Zong W, Petersen K, Shah NM, Brandon JH, et al. (2021): Circadian- and sex-dependent increases in intravenous cocaine self-administration in *Npas2* mutant mice. *J Neurosci* 41:1046–1058.
- McClung CA (2013): How might circadian rhythms control mood? Let me count the ways. *Biol Psychiatry* 74:242–249.
- Logan RW, McClung CA (2019): Rhythms of life: Circadian disruption and brain disorders across the lifespan. *Nat Rev Neurosci* 20:49–65.
- Ketchesin KD, Becker-Krail D, McClung CA (2020): Mood-related central and peripheral clocks. *Eur J Neurosci* 51:326–345.
- Walker WH 2nd, Walton JC, DeVries AC, Nelson RJ (2020): Circadian rhythm disruption and mental health. *Transl Psychiatry* 10:28.
- Mannella F, Gurney K, Baldassarre G (2013): The nucleus accumbens as a nexus between values and goals in goal-directed behavior: A review and a new hypothesis. *Front Behav Neurosci* 7:135.
- Scotfield MD, Heinsbroek JA, Gipson CD, Kupchik YM, Spencer S, Smith ACW, et al. (2016): The nucleus accumbens: Mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis. *Pharmacol Rev* 68:816–871.
- Scotfield MD, Kalivas PW (2014): Astrocytic dysfunction and addiction: Consequences of impaired glutamate homeostasis. *Neuroscientist* 20:610–622.
- Kim R, Healey KL, Sepulveda-Orengo MT, Reissner KJ (2018): Astroglial correlates of neuropsychiatric disease: From astrocytopathy to astrogliosis. *Prog Neuropsychopharmacol Biol Psychiatry* 87:126–146.
- Sofroniew MV, Vinters HV (2010): Astrocytes: Biology and pathology. *Acta Neuropathol* 119:7–35.
- Danbolt NC (2001): Glutamate uptake. *Prog Neurobiol* 65:1–105.
- Malarkey EB, Pappas V (2008): Mechanisms of glutamate release from astrocytes. *Neurochem Int* 52:142–154.
- Bolaños JP (2016): Bioenergetics and redox adaptations of astrocytes to neuronal activity. *J Neurochem* 139(suppl 2):115–125.
- Gonçalves CA, Rodrigues L, Bobermin LD, Zanotto C, Vizuete A, Quincozes-Santos A, et al. (2019): Glycolysis-derived compounds from astrocytes that modulate synaptic communication. *Front Neurosci* 12:1035.
- Prolo LM, Takahashi JS, Herzog ED (2005): Circadian rhythm generation and entrainment in astrocytes. *J Neurosci* 25:404–408.

43. Yagita K, Yamanaka I, Emoto N, Kawakami K, Shimada S (2010): Real-time monitoring of circadian clock oscillations in primary cultures of mammalian cells using Tol2 transposon-mediated gene transfer strategy. *BMC Biotechnol* 10:3.
44. Chi-Castañeda D, Ortega A (2016): Clock genes in glia cells: A rhythmic history. *ASN Neuro* 8:1759091416670766.
45. Gwak YS, Kang J, Leem JW, Hulsebosch CE (2007): Spinal AMPA receptor inhibition attenuates mechanical allodynia and neuronal hyperexcitability following spinal cord injury in rats. *J Neurosci Res* 85:2352–2359.
46. Beaulé C, Swanson A, Leone MJ, Herzog ED (2009): Circadian modulation of gene expression, but not glutamate uptake, in mouse and rat cortical astrocytes. *PLoS One* 4:e7476.
47. Marpegan L, Swanson AE, Chung K, Simon T, Haydon PG, Khan SK, *et al.* (2011): Circadian regulation of ATP release in astrocytes. *J Neurosci* 31:8342–8350.
48. Brancaccio M, Patton AP, Chesham JE, Maywood ES, Hastings MH (2017): Astrocytes control circadian timekeeping in the suprachiasmatic nucleus via glutamatergic signaling. *Neuron* 93:1420–1435. e5.
49. Tso CF, Simon T, Greenlaw AC, Puri T, Mieda M, Herzog ED (2017): Astrocytes regulate daily rhythms in the suprachiasmatic nucleus and behavior. *Curr Biol* 27:1055–1061.
50. Brancaccio M, Edwards MD, Patton AP, Smyllie NJ, Chesham JE, Maywood ES, Hastings MH (2019): Cell-autonomous clock of astrocytes drives circadian behavior in mammals. *Science* 363:187–192.
51. Kalivas PW (2009): The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci* 10:561–572.
52. Reissner KJ, Gipson CD, Tran PK, Knackstedt LA, Scofield MD, Kalivas PW (2015): Glutamate transporter GLT-1 mediates N-acetylcysteine inhibition of cocaine reinstatement. *Addict Biol* 20:316–323.
53. Scofield MD, Boger HA, Smith RJ, Li H, Haydon PG, Kalivas PW (2015): Gq-DREADD selectively initiates glial glutamate release and inhibits cue-induced cocaine seeking. *Biol Psychiatry* 78:441–451.
54. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, *et al.* (2008): A transcriptome database for astrocytes, neurons, and oligodendrocytes: A new resource for understanding brain development and function. *J Neurosci* 28:264–278.
55. Yang Y, Vidensky S, Jin L, Jie C, Lorenzini I, Frankl M, Rothstein JD (2011): Molecular comparison of GLT1+ and ALDH1L1+ astrocytes in vivo in astroglial reporter mice. *Glia* 59:200–207.
56. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O’Keefe S, *et al.* (2014): An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex [published correction appears in *J Neurosci* 2015; 35:846]. *J Neurosci* 34:11929–11947.
57. Storch KF, Paz C, Signorovitch J, Raviola E, Pawlyk B, Li T, Weitz CJ (2007): Intrinsic circadian clock of the mammalian retina: Importance for retinal processing of visual information. *Cell* 130:730–741.
58. Sanz E, Yang L, Su T, Morris DR, McKnight GS, Amieux PS (2009): Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. *Proc Natl Acad Sci U S A* 106:13939–13944.
59. Chandra R, Francis TC, Konkalmatt P, Amgalan A, Gancarz AM, Dietz DM, Lobo MK (2015): Opposing role for *Egr3* in nucleus accumbens cell subtypes in cocaine action. *J Neurosci* 35:7927–7937.
60. Hughes ME, Hogenesch JB, Kornacker K (2010): JTK\_CYCLE: An efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets. *J Biol Rhythms* 25:372–380.
61. Wu G, Zhu J, Yu J, Zhou L, Huang JZ, Zhang Z (2014): Evaluation of five methods for genome-wide circadian gene identification. *J Biol Rhythms* 29:231–242.
62. Krämer A, Green J, Pollard J Jr, Tugendreich S (2014): Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics* 30:523–530.
63. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, *et al.* (2019): Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* 10:1523.
64. Haskew-Layton RE, Payappilly JB, Smirnova NA, Ma TC, Chan KK, Murphy TH, *et al.* (2010): Controlled enzymatic production of astrocytic hydrogen peroxide protects neurons from oxidative stress via an Nrf2-independent pathway. *Proc Natl Acad Sci U S A* 107:17385–17390.
65. Baxter PS, Bell KFS, Hasel P, Kaindl AM, Fricker M, Thomson D, *et al.* (2015): Synaptic NMDA receptor activity is coupled to the transcriptional control of the glutathione system [published correction appears in *Nat Commun* 2017; 8:16158]. *Nat Commun* 6:6761.
66. Lerchundi R, Fernández-Moncada I, Contreras-Baeza Y, Sotelo-Hitschfeld T, Mächler P, Wyss MT, *et al.* (2015): NH4(+) triggers the release of astrocytic lactate via mitochondrial pyruvate shunting. *Proc Natl Acad Sci U S A* 112:11090–11095.
67. Muraleedharan R, Gawali MV, Tiwari D, Sukumaran A, Oatman N, Anderson J, *et al.* (2020): AMPK-regulated astrocytic lactate shuttle plays a non-cell-autonomous role in neuronal survival. *Cell Rep* 32:108092.
68. Logan RW, Edgar N, Gillman AG, Hoffman D, Zhu X, McClung CA (2015): Chronic stress induces brain region-specific alterations of molecular rhythms that correlate with depression-like behavior in mice. *Biol Psychiatry* 78:249–258.
69. Brami-Cherrier K, Lewis RG, Cervantes M, Liu Y, Tognini P, Baldi P, *et al.* (2020): Cocaine-mediated circadian reprogramming in the striatum through dopamine D2R and PPAR $\gamma$  activation. *Nat Commun* 11:4448.
70. Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, *et al.* (2008): Application of a translational profiling approach for the comparative analysis of CNS cell types [published correction appears in *Cell* 2009; 139:1022]. *Cell* 135:749–1762.
71. Sakers K, Lake AM, Khazanchi R, Ouwenga R, Vasek MJ, Dani A, Dougherty JD (2017): Astrocytes locally translate transcripts in their peripheral processes. *Proc Natl Acad Sci U S A* 114:E3830–E3838.
72. Sapkota D, Lake AM, Yang W, Yang C, Wesseling H, Guise A, *et al.* (2019): Cell-type-specific profiling of alternative translation identifies regulated protein isoform variation in the mouse brain. *Cell Rep* 26:594–607. e7.
73. Clarke LE, Liddel SA, Chakraborty C, Münch AE, Heiman M, Barres BA (2018): Normal aging induces A1-like astrocyte reactivity. *Proc Natl Acad Sci U S A* 115:E1896–E1905.
74. McKenzie AT, Wang M, Hauberg ME, Fullard JF, Kozlenkov A, Keenan A, *et al.* (2018): Brain cell type specific gene expression and co-expression network architectures [published correction appears in *Sci Rep* 2021; 11:19430]. *Sci Rep* 8:8868.
75. Pan J, Ma N, Yu B, Zhang W, Wan J (2020): Transcriptomic profiling of microglia and astrocytes throughout aging. *J Neuroinflammation* 17:97.
76. Lananna BV, Nadarajah CJ, Izumo M, Cedeño MR, Xiong DD, Dimitry J, *et al.* (2018): Cell-autonomous regulation of astrocyte activation by the circadian clock protein BMAL1. *Cell Rep* 25:1–9. e5.
77. Stead JDH, Clinton S, Neal C, Schneider J, Jama A, Miller S, *et al.* (2006): Selective breeding for divergence in novelty-seeking traits: Heritability and enrichment in spontaneous anxiety-related behaviors. *Behav Genet* 36:697–712.
78. Flagel SB, Robinson TE, Clark JJ, Clinton SM, Watson SJ, Seeman P, *et al.* (2010): An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: Implications for addiction. *Neuropsychopharmacology* 35:388–400.
79. Zhou Z, Blandino P, Yuan Q, Shen PH, Hodgkinson CA, Virkkunen M, *et al.* (2019): Exploratory locomotion, a predictor of addiction vulnerability, is oligogenic in rats selected for this phenotype. *Proc Natl Acad Sci U S A* 116:13107–13115.
80. Bush DEA, Vaccarino FJ (2007): Individual differences in elevated plus-maze exploration predicted progressive-ratio cocaine self-administration break points in Wistar rats. *Psychopharmacology (Berl)* 194:211–219.
81. Flagel SB, Waselus M, Clinton SM, Watson SJ, Akil H (2014): Antecedents and consequences of drug abuse in rats selectively bred for

- high and low response to novelty. *Neuropharmacology* 76 Pt B:425–436.
82. Dickson PE, Ndikum J, Wilcox T, Clark J, Roy B, Zhang L, *et al.* (2015): Association of novelty-related behaviors and intravenous cocaine self-administration in diversity outbred mice. *Psychopharmacology (Berl)* 232:1011–1024.
  83. Wingo T, Nesil T, Choi JS, Li MD (2016): Novelty seeking and drug addiction in humans and animals: From behavior to molecules. *J Neuroimmune Pharmacol* 11:456–470.
  84. Guilloux JP, Seney M, Edgar N, Sibille E (2011): Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: Relevance to emotionality and sex. *J Neurosci Methods* 197:21–31.
  85. Mishima N, Higashitani F, Teraoka K, Yoshioka R (1986): Sex differences in appetitive learning of mice. *Physiol Behav* 37:263–268.
  86. van Haaren F, van Hest A, Heinsbroek RP (1990): Behavioral differences between male and female rats: Effects of gonadal hormones on learning and memory. *Neurosci Biobehav Rev* 14:23–33.
  87. McDowell AL, Heath KM, Garrahy PE (2013): The effects of sex and chronic restraint on instrumental learning in rats. *Neurosci J* 2013:893126.
  88. Liddelow SA, Barres BA (2017): Reactive astrocytes: Production, function, and therapeutic potential. *Immunity* 46:957–967.
  89. Vomund S, Schäfer A, Parnham MJ, Brüne B, von Knethen A (2017): Nrf2, the master regulator of anti-oxidative responses. *Int J Mol Sci* 18:2772.
  90. Bélanger M, Allaman I, Magistretti PJ (2011): Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 14:724–738.
  91. Barca-Mayo O, Pons-Espinal M, Follert P, Armirotti A, Berdondini L, De Pietri Tonelli D (2017): Astrocyte deletion of Bmal1 alters daily locomotor activity and cognitive functions via GABA signalling. *Nat Commun* 8:14336.
  92. Fellin T, D'Ascenzo M, Haydon PG (2007): Astrocytes control neuronal excitability in the nucleus accumbens. *ScientificWorldJournal* 7:89–97.
  93. Parpura V, Verkhratsky A (2012): Astrocytes revisited: Concise historic outlook on glutamate homeostasis and signaling. *Croat Med J* 53:518–528.
  94. Parekh PK, Becker-Krail D, Sundaravelu P, Ishigaki S, Okado H, Sobue G, *et al.* (2018): Altered GluA1 (Gria1) function and accumbal synaptic plasticity in the ClockΔ19 model of bipolar mania. *Biol Psychiatry* 84:817–826.
  95. Pellerin L, Magistretti PJ (1994): Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A* 91:10625–10629.
  96. Magistretti PJ, Pellerin L (1996): Cellular bases of brain energy metabolism and their relevance to functional brain imaging: Evidence for a prominent role of astrocytes. *Cereb Cortex* 6:50–61.
  97. Pellerin L, Magistretti PJ (2012): Sweet sixteen for ANLS. *J Cereb Blood Flow Metab* 32:1152–1166.
  98. Magistretti PJ, Allaman I (2018): Lactate in the brain: From metabolic end-product to signalling molecule. *Nat Rev Neurosci* 19:235–249.
  99. Ahlersová E, Ahlers I, Toropila M, Smajda B, Datelinka I (1981): Circadian rhythm of the lactate and pyruvate concentration in rat liver and blood. *Physiol Bohemoslov* 30:213–220.
  100. Rutter J, Reick M, Wu LC, McKnight SL (2001): Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293:510–514.
  101. Naylor E, Aillon DV, Barrett BS, Wilson GS, Johnson DA, Johnson DA, *et al.* (2012): Lactate as a biomarker for sleep. *Sleep* 35:1209–1222.
  102. Henriksson E, Huber AL, Soto EK, Kriebs A, Vaughan ME, Duglan D, *et al.* (2017): The liver circadian clock modulates biochemical and physiological responses to metformin. *J Biol Rhythms* 32:345–358.
  103. Miličević N, Ten Brink JB, Ten Asbroek ALMA, Bergen AA, Felder-Schmittbuhl MP (2020): The circadian clock regulates RPE-mediated lactate transport via SLC16A1 (MCT1). *Exp Eye Res* 190:107861.
  104. Wallace NK, Pollard F, Savenkova M, Karatsoreos IN (2020): Effect of aging on daily rhythms of lactate metabolism in the medial prefrontal cortex of male mice. *Neuroscience* 448:300–310.
  105. Freyberg Z, Logan RW (2018): The intertwined roles of circadian rhythms and neuronal metabolism fueling drug reward and addiction. *Curr Opin Physiol* 5:80–89.
  106. Boury-Jamot B, Carrard A, Martin JL, Halfon O, Magistretti PJ, Boutrel B (2016): Disrupting astrocyte-neuron lactate transfer persistently reduces conditioned responses to cocaine. *Mol Psychiatry* 21:1070–1076.
  107. Boury-Jamot B, Halfon O, Magistretti PJ, Boutrel B (2016): Lactate release from astrocytes to neurons contributes to cocaine memory formation. *Bioessays* 38:1266–1273.
  108. Zhang Y, Xue Y, Meng S, Luo Y, Liang J, Li J, *et al.* (2016): Inhibition of lactate transport erases drug memory and prevents drug relapse. *Biol Psychiatry* 79:928–939.
  109. Skupio U, Tertli M, Bilecki W, Barut J, Korostynski M, Golda S, *et al.* (2020): Astrocytes determine conditioned response to morphine via glucocorticoid receptor-dependent regulation of lactate release. *Neuropsychopharmacology* 45:404–415.
  110. Perreau-Lenz S, Spanagel R (2015): Clock genes × stress × reward interactions in alcohol and substance use disorders. *Alcohol* 49:351–357.
  111. Becker-Krail D, McClung C (2016): Implications of circadian rhythm and stress in addiction vulnerability. *F1000Res* 5:59.
  112. Allaman I, Pellerin L, Magistretti PJ (2004): Glucocorticoids modulate neurotransmitter-induced glycogen metabolism in cultured cortical astrocytes. *J Neurochem* 88:900–908.
  113. Alam MM, Okazaki K, Nguyen LTT, Ota N, Kitamura H, Murakami S, *et al.* (2017): Glucocorticoid receptor signaling represses the anti-oxidant response by inhibiting histone acetylation mediated by the transcriptional activator NRF2. *J Biol Chem* 292:7519–7530.
  114. Habas A, Hahn J, Wang X, Margeta M (2013): Neuronal activity regulates astrocytic Nrf2 signaling. *Proc Natl Acad Sci U S A* 110:18291–18296.
  115. Jimenez-Blasco D, Santofimia-Castaño P, Gonzalez A, Almeida A, Bolaños JP (2015): Astrocyte NMDA receptors' activity sustains neuronal survival through a Cdk5-Nrf2 pathway. *Cell Death Differ* 22:1877–1889.
  116. McGann JC, Mandel G (2018): Neuronal activity induces glutathione metabolism gene expression in astrocytes. *Glia* 66:2024–2039.
  117. Ma Q (2013): Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 53:401–426.
  118. Baxter PS, Hardingham GE (2016): Adaptive regulation of the brain's antioxidant defences by neurons and astrocytes. *Free Radic Biol Med* 100:147–152.
  119. Tu W, Wang H, Li S, Liu Q, Sha H (2019): The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases. *Aging Dis* 10:637–651.
  120. Schieber M, Chandel NS (2014): ROS function in redox signaling and oxidative stress. *Curr Biol* 24:R453–R462.
  121. Berríos-Cárcamo P, Quezada M, Quintanilla ME, Morales P, Ezquer M, Herrera-Marschitz M, *et al.* (2020): Oxidative stress and neuroinflammation as a pivot in drug abuse. A focus on the therapeutic potential of antioxidant and anti-inflammatory agents and biomolecules. *Antioxidants (Basel)* 9:830.
  122. Pavlek LR, Dillard J, Rogers LK (2020): The role of oxidative stress in toxicities due to drugs of abuse. *Curr Opin Toxicol* 20(21):29–35.
  123. Garcia JA, Zhang D, Estill SJ, Michonoff C, Rutter J, Reick M, *et al.* (2000): Impaired cued and contextual memory in NPAS2-deficient mice. *Science* 288:2226–2230.