Integrating Circadian Rhythm and Neurobiology in Precision Medicine: A Novel Approach for Treating Severe Autism Through the Exploration of Time Cells and Genetic Mechanisms

Peter De Ceuster

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Abstract

Autism Spectrum Disorder (ASD) represents a highly heterogeneous group of neurodevelopmental conditions defined by core symptoms of impaired social communication and restricted, repetitive behaviors. Severe autism cases, which often include intellectual disability, epilepsy, and profound functional impairments, remain particularly resistant to existing therapeutic interventions. Recent genomic discoveries—many spearheaded by Silvia De Rubeis and colleagues—have highlighted the critical role of synaptic, transcriptional, and chromatin regulatory pathways in ASD. These insights have shifted focus toward a mechanistic understanding of ASD at the molecular level.

At the same time, circadian biology has emerged as a central regulator of neurodevelopment, synaptic plasticity, and behavior. Circadian rhythms, orchestrated by transcriptional–translational feedback loops (TTFLs) in the suprachiasmatic nucleus (SCN), regulate thousands of genes involved in metabolism, neurotransmission, and DNA repair. Disruption of these rhythms exacerbates excitatory–inhibitory imbalance, a hallmark of ASD pathophysiology.

The convergence of circadian regulation, time cell dynamics, and ASD genetics creates a promising landscape for therapeutic innova-

tion. A precision medicine framework that incorporates circadian biology could enable the rational design of novel drugs targeting oscillatory mechanisms at the molecular level.

Neurobiological Basis of Circadian Rhythm

The SCN synchronizes peripheral and central clocks via signaling molecules such as vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP). These oscillatory signals entrain downstream neuronal populations, influencing neurotransmitter systems including GABA, glutamate, dopamine, and serotonin.

In ASD, disrupted SCN signaling manifests as altered sleep–wake cycles, diminished melatonin secretion, and abnormal cortical oscillations. Melatonin pathway disruption is particularly significant, as many ASD patients carry polymorphisms in the AANAT or ASMT genes, which encode enzymes in melatonin synthesis. At the molecular level, melatonin acts via MT1 and MT2 G-protein coupled receptors to modulate cAMP/PKA signaling and Ca2+ channel activity, both of which influence synaptic transmission. Drug candidates that act as MT2-selective agonists (e.g., tasimelteon) may offer circadian phase-specific correction of excitatory–inhibitory imbalance in ASD.

Neurotransmitter regulation by circadian rhythms is also tightly coupled with molecular action. For instance, CLOCK-BMAL1 heterodimers regulate expression of tyrosine hydroxylase, controlling dopamine synthesis. Given dopamine's role in social motivation, drugs aligning dopaminergic rhythms with SCN output could have therapeutic potential.

Molecular Mechanisms of Circadian Rhythm Regulation

At the molecular core, the circadian clock is built upon transcriptional–translational feedback loops (TTFLs). CLOCK and BMAL1 drive expression of PER and CRY genes, whose proteins translocate to the nucleus to inhibit CLOCK-BMAL1 activity. This cycle is finely tuned by post-translational modifications:

Casein kinase 1/ phosphorylates PER proteins, regulating their nuclear entry and degradation. AMPK phosphorylates CRY, linking energy metabolism to circadian rhythm. SIRT1 deacetylates histones and PER2, integrating NAD+-dependent metabolic cycles with circadian regulation. Disruption of these loops has profound effects on synaptic protein expression and neuronal excitability. ASD-associated genes such as CHD8 (a chromatin remodeler) interact directly with circadian transcriptional regulators, altering chromatin accessibility of CLOCK/BMAL1 targets.

Drug discovery efforts have begun to exploit these mechanisms. Small molecule CK1/ inhibitors (e.g., PF-670462) shift circadian phases and restore PER protein turnover. REV-ERB agonists (SR9009, SR9011) increase circadian amplitude and synchronize metabolic and synaptic oscillations. SIRT1 activators (resveratrol analogues) restore redox-sensitive circadian function, potentially correcting synaptic homeostasis in ASD subtypes with mitochondrial dysfunction.

The molecular regulation of circadian rhythms involves a tightly coupled transcriptional–translational feedback loop (TTFL) that integrates post-translational modifications, chromatin remodeling, and metabolite sensing. While the core CLOCK–BMAL1 heterodimer drives the rhythmic transcription of PER and CRY genes, multiple accessory loops contribute to the robustness of oscillations. These include the REV-ERB/ and ROR// nuclear receptors, which repress or activate Bmal1 transcription respectively, estab-

lishing antiphase oscillations critical for system stability.

Recent structural biology studies reveal that PER proteins form multiprotein complexes with CRY, casein kinase 1/ (CK1/), and other scaffolding proteins. Cryo-EM reconstructions indicate that CK1 docks onto the C-terminal tail of PER2, phosphorylating serine residues that serve as phosphodegrons, targeting the complex for ubiquitination and proteasomal degradation. This phosphorylation-mediated turnover acts as the primary timing mechanism, setting the circadian period. Small molecule inhibitors of CK1/ not only lengthen the circadian period but also alter downstream gene expression in synaptic and metabolic pathways, suggesting potential application for ASD where circadian misalignment exacerbates excitatory–inhibitory imbalance.

Epigenetic regulation plays an equally important role. Histone acetyltransferase p300 interacts with CLOCK, acetylating both histones and BMAL1 itself. Acetylation enhances transcriptional activation at E-box elements. SIRT1, a NAD+-dependent deacetylase, counteracts this by deacetylating BMAL1 and PER2, linking energy status with circadian gene expression. Dysregulated NAD+/SIRT1 activity has been documented in mitochondrial dysfunction associated with ASD. Thus, pharmacological activation of SIRT1 could reset circadian amplitude and simultaneously improve metabolic resilience in ASD neurons.

In parallel, chromatin remodelers such as CHD8, a high-confidence ASD gene, regulate circadian transcription. Knockdown of CHD8 in human neurons alters accessibility at enhancers bound by CLOCK–BMAL1, flattening circadian expression of metabolic and synaptic genes. This provides a direct mechanistic link between ASD genetics and circadian dysfunction. Drug strategies that enhance CHD8 function or mimic its chromatin-opening role could restore circadian-driven transcriptional waves.

Metabolic sensors including AMPK and mTOR also intersect with circadian regulators. AMPK phosphorylates CRY1, promoting degradation and

accelerating circadian phase. In neurons, mTOR signaling is rhythmic and regulates synaptic translation, intersecting with ASD-associated pathways. Inhibitors of mTOR (e.g., rapamycin analogues) could be chronopharmacologically applied to align with circadian phases of peak translational dysregulation, minimizing side effects while maximizing synaptic correction.

At the systems level, circadian genes regulate over 40 percent of the transcriptome in a time-of-day–dependent manner. This rhythmic transcription includes synaptic scaffolding proteins, ion channel subunits, and neurotransmitter receptors, all implicated in ASD. Disruption of circadian regulators thus disturbs excitatory–inhibitory homeostasis. Identifying which circadian-controlled synaptic proteins are most altered in ASD subtypes will allow for targeted small molecule or gene therapy development.

The Role of Time Cells in Neurobiology

Time cells in the hippocampus and medial prefrontal cortex represent sequentially firing neurons that encode temporal structure during cognitive tasks. Their activity is dependent on:

NMDA receptor–dependent plasticity and calcium influx. cAMP/PKA–CREB signaling cascades regulating gene transcription. Synchronization with circadian oscillations in the SCN and entorhinal cortex. In ASD, disrupted NMDA receptor signaling and impaired interneuron synchronization compromise time cell activity, leading to deficits in episodic memory and social timing. These impairments are exacerbated by circadian misalignment.

Targeted pharmacological interventions could enhance time cell fidelity:

Positive allosteric modulators of NMDA receptors (glycine site agonists, D-serine analogs). Subtype-specific GABAA receptor modulators to restore parvalbumin interneuron function. Chronopharmacological delivery of drugs to coincide with peak CREB phosphorylation, enhancing time cell plasticity. Time cells in the hippocampus and medial prefrontal cortex represent a

fundamental mechanism for encoding temporal context in episodic memory and social interaction. These neurons fire in sequential patterns that bridge gaps between discrete events, allowing the brain to organize experience along a temporal framework. Their function depends on multiple molecular and circuit-level mechanisms.

At the receptor level, NMDA receptor-mediated calcium influx is essential for time cell plasticity. Activation of CaMKII and downstream phosphorylation of CREB regulate gene transcription required for the maintenance of temporal coding. Importantly, CREB activation is modulated by circadian oscillations in kinase activity, meaning that the fidelity of time cell sequences depends on circadian phase. In ASD, impaired NMDA receptor signaling (e.g., due to GRIN2B mutations) disrupts calcium-dependent gene expression, leading to degraded time cell firing sequences. Pharmacological strategies enhancing NMDA receptor co-agonist binding (glycine transporter inhibitors, D-serine analogues) could restore proper temporal coding.

GABAergic interneurons, particularly parvalbumin-positive basket cells, synchronize hippocampal oscillations necessary for time cell sequences. Circadian regulation of GABA release is mediated by CLOCK-controlled transcription of GAD1 and GAD2 (enzymes for GABA synthesis). In ASD, reduced interneuron function contributes to noisy or desynchronized time cell activity. Targeting GABAA receptor subtypes with selective positive allosteric modulators timed to circadian oscillations could restore synchrony and improve temporal coding.

Dopaminergic input from the ventral tegmental area modulates reward prediction signals in time cell networks. Circadian regulation of dopamine synthesis via tyrosine hydroxylase ensures rhythmic dopamine availability. In ASD models, blunted dopamine release impairs reinforcement learning and disrupts time cell reliability. Agents targeting dopamine D1/D2 receptor balance, delivered in circadian alignment, might enhance both reward processing and time cell stability.

At the molecular scale, immediate early genes such as Arc, c-Fos, and Egr1 are rhythmically expressed and necessary for time cell plasticity. Epigenomic studies indicate that time cells rely on rhythmic chromatin accessibility, which is disrupted in ASD by mutations in chromatin regulators like CHD8 and ARID1B. Pharmacological agents modulating histone acetylation (HDAC inhibitors) in circadian-phase–specific dosing schedules could restore proper gene induction and strengthen time cell performance.

The therapeutic implications are profound. If time cell activity can be rescued pharmacologically, then deficits in episodic memory, social timing, and sequence learning in ASD may improve. Early experiments with optogenetic modulation of time cells in mouse models show that restoring sequential firing patterns rescues temporal learning tasks. Translating this into pharmacological precision medicine will require identifying molecular entry points—NMDA receptor modulation, circadian kinase targeting, or chromatin remodeling interventions—combined with chronotherapy.

Genetic Mechanisms of ASD

De Rubeis and colleagues have identified convergent genetic risk factors for ASD, including:

SHANK3: Scaffold protein at excitatory synapses. Loss disrupts AMPA/NMDA receptor localization. SYNGAP1: GTPase-activating protein controlling Ras–MAPK signaling. Haploinsufficiency causes hyperexcitable synapses. CHD8: Chromatin remodeler regulating circadian-linked transcriptional networks. FOXP1/2: Transcription factors with rhythmic expression, tied to language development. Many of these genes exhibit circadian regulation. For example, SHANK3 transcript levels oscillate across the circadian cycle in cortical neurons, while CHD8 mutations alter circadian gene expression in stem cell–derived neurons.

This genetic–circadian interplay highlights a therapeutic window: timing

interventions to circadian phases when mutated genes exert maximal impact could amplify drug efficacy. For example, targeting excitatory synapses in SHANK3-haploinsufficient models with mGluR5 antagonists during peak glutamatergic oscillations may correct excitatory–inhibitory imbalance more effectively than constant dosing.

The genetic architecture of ASD includes both rare high-penetrance mutations and common polygenic variation. Several high-confidence ASD risk genes converge on pathways regulating synaptic function, transcription, and chromatin remodeling. Importantly, many of these genes display circadian regulation or intersect directly with molecular clock machinery.

SHANK3, a scaffolding protein at excitatory synapses, interacts with NMDA and AMPA receptor complexes. Loss-of-function mutations lead to disrupted glutamatergic transmission. Intriguingly, SHANK3 expression oscillates across the circadian cycle, suggesting that time-of-day may influence synaptic vulnerability in SHANK3-deficient individuals. Pharmacological strategies that stabilize AMPA receptor trafficking, administered during circadian peaks of glutamatergic activity, could improve synaptic strength in SHANK3 haploinsufficiency.

SYNGAP1 encodes a Ras GTPase-activating protein that regulates MAPK and PI3K signaling downstream of NMDA receptor activation. Haploinsufficiency causes excessive ERK signaling and hyperexcitability. Circadian regulation of Ras–MAPK signaling has been documented in the hippocampus, with peak ERK activity occurring during active phases. Timed inhibition of ERK signaling with MEK inhibitors could normalize excitatory–inhibitory balance in SYNGAP1-related ASD.

CHD8 mutations represent a paradigmatic link between chromatin remodeling and circadian dysfunction. As a regulator of transcriptional accessibility, CHD8 controls the rhythmic expression of hundreds of genes, including those involved in neurodevelopment and synaptic signaling. Loss of CHD8 function flattens rhythmic transcription, disrupting daily waves of synaptic

protein synthesis. This may explain the severe sleep and behavioral phenotypes seen in CHD8-mutant ASD. Pharmacological interventions aimed at enhancing circadian amplitude (e.g., REV-ERB agonists) may counterbalance the flattening effect of CHD8 mutations.

FMR1, the gene mutated in fragile X syndrome, regulates mRNA translation at synapses. FMRP expression shows circadian oscillation, and fragile X models exhibit disrupted circadian behavior. Drugs targeting mGluR5 signaling, already explored in fragile X, might be more effective if administered in circadian alignment with FMRP oscillations. This represents a precision approach that could also apply to other ASD subtypes with translational dysregulation.

Beyond single-gene mutations, polygenic influences affect circadian pathways. Genome-wide association studies reveal enrichment of ASD-associated SNPs near circadian regulators such as PER2, NR1D1, and ARNTL (BMAL1). This suggests that common variants disrupting circadian amplitude may increase ASD risk at the population level. Identifying circadian genotypes may allow stratification of patients for circadian-targeted therapies.

Future drug discovery should integrate gene–circadian interactions into screening pipelines. High-throughput assays could test small molecules against circadian-regulated targets in neuronal cultures derived from ASD patients with defined genetic backgrounds. This approach will enable the rational design of drugs that restore both synaptic and circadian homeostasis, tailored to genetic subtypes.

Integration of Circadian Rhythm and Neurobiology in Precision Medicine

Precision medicine aims to tailor therapy to individual genetic and physiological contexts. Integrating circadian biology requires:

Chronotherapy: Delivering drugs at circadian phases aligned with target

expression. Biomarker monitoring: Using melatonin onset, body temperature, or transcriptomic markers to define circadian state. Genomic diagnostics: Identifying circadian-linked ASD mutations (e.g., CHD8, PER2). Drug repurposing: Aligning existing drugs with circadian pharmacokinetics. Candidate interventions include:

CK1/ inhibitors to restore PER protein turnover. REV-ERB agonists to enhance circadian amplitude and suppress excitatory hyperactivity. Subtype-specific GABAA modulators to reestablish inhibitory balance in ASD. CRISPR-based gene correction of SHANK3 or CHD8, timed with circadian peaks in DNA repair activity for higher editing efficiency.

Case Studies and Future Perspectives

Rodent SHANK3 model: Circadian disruption worsens repetitive grooming behaviors. Restoring circadian alignment with light entrainment improves social exploration.

SYNGAP1 haploinsufficient mice: Exhibit disrupted circadian gene expression and impaired time cell coding. NMDA receptor modulators partially restore cognitive performance.

Clinical melatonin trials: Demonstrate modest improvement in sleep and social responsiveness, though effect sizes are small. These trials underscore the importance of developing more potent and selective circadian-targeted drugs.

Future directions include circadian-phase–specific drug screening, high-throughput identification of small molecules targeting TTFL regulators, and use of time cell activity as a biomarker for therapeutic efficacy. Ultimately, integrating circadian neurobiology with genetic diagnostics may provide a path to novel, mechanism-based therapies for severe autism.

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