Stroke

TOPICAL REVIEW

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Circadian Biology and Stroke

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ABSTRACT: Circadian biology modulates almost all aspects of mammalian physiology, disease, and response to therapies. Emerging data suggest that circadian biology may significantly affect the mechanisms of susceptibility, injury, recovery, and the response to therapy in stroke. In this review/perspective, we survey the accumulating literature and attempt to connect molecular, cellular, and physiological pathways in circadian biology to clinical consequences in stroke. Accounting for the complex and multifactorial effects of circadian rhythm may improve translational opportunities for stroke diagnostics and therapeutics.

Key Words: biomarkers ■ circadian rhythm ■ immune system ■ ischemia ■ neuroprotection ■ sleep

Clinical trials of neuroprotection mostly recruit patients in the daytime. For diurnal humans, this is their awake active phase. Experiments in mouse and rat models of cerebral ischemia are also usually performed in the daytime. However, for nocturnal rodents, this is their sleep and inactive phase, providing a complication when extrapolating from animal models to humans.[1] A recent study hypothesized that some of the difficulties in translating stroke targets from the laboratory into the clinic may be potentially related in part to a circadian mismatch between animal models and clinical trials of neuroprotection.[2]

The mammalian circadian system is composed of a master oscillator in the hypothalamic suprachiasmatic nucleus and circadian oscillators in all organs throughout the body, including heart, kidney, and blood vessels. These central and peripheral oscillators generate cell-autonomous rhythms based on transcriptional/translational feedback loops of multiple clock genes, including Per1, Per2, Clock, and Bmal1. This multi-oscillator system generates endogenous circadian rhythms (ie, even absent environmental or behavioral rhythms) in all physiological systems, including cardiovascular, metabolic, immune, and inflammatory function.[3] Breakdown of this network leads to internal desynchrony and circadian disruption, which is frequently a hallmark of disease.[4]

Circadian rhythms are now recognized to modulate the response of heart tissue to ischemia.[3] Hence, circadian effects will likely influence stroke mechanisms and targets. In this review/perspective, we survey existing literature on circadian effects in clinical and experimental stroke research, highlight gaps in knowledge, and discuss the implications and opportunities for translational advance.

CLINICAL PROFILE OF DIURNAL EFFECTS IN STROKE TIMING AND TREATMENT

Observed rhythms in humans are usually not purely circadian, but diurnal—the combination of both endogenous circadian rhythms and behaviors such as sleep, eating, activity, and posture changes. Circadian misalignment, arising from shift work, jet lag, weekday-weekend activity shifts (social jetlag), or circadian sleep-wake disturbances, increases cardiovascular risk factors.[5] Circadian

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misalignment is associated with lower high-density lipoprotein-cholesterol levels, higher triglyceride levels, disrupted cortisol rhythms, increased C-reactive protein, increased blood pressure, and prediabetic states with decreased insulin sensitivity and higher glucose levels, all of which are likely predispose to stroke occurrence.[6–9]

Diurnal variation in first detection of stroke symptoms has been documented across diverse geographies and race-ethnic groups.[10] A meta-analysis of 31 studies collectively reporting 11 816 patients with ischemic stroke, hemorrhagic stroke, and transient ischemic attack found first detection occurred between 6 AM and 12 noon in 37%, between 12 noon and 6 PM in 26%, between 6 PM and 12 midnight in 19%, and between 12 midnight and 6 AM in 18%.[10] The morning surge in first detection was more pronounced for ischemic stroke and transient ischemic attack than for hemorrhagic stroke. Among ischemic strokes, the same pattern of increased morning first detection occurs for each of the major mechanistic subtypes of large artery atherosclerotic, cardioembolic, small vessel disease, and cryptogenic.[11,12] In both intracerebral hemorrhage (ICH) and subarachnoid hemorrhage, there is a bimodal rhythm of first detection, with the highest peak in morning and second peak in early afternoon/evening.[13,14]

These variations in timing of first detection documented in epidemiological and large cohort studies likely reflect both a genuine circadian variation in biological onset of stroke but also a confounding effect arising from wake-up strokes, for which the time symptoms are first observed may not reflect the time of actual stroke onset. Therefore, wake-up strokes cause a shift in time of stroke discovery from night to morning. Since wake-up strokes account for 8% to 28% of all ischemic strokes, they contribute importantly to the clustering of first symptom observation in mid-morning.[15] Nonetheless, even after accounting for wake-up strokes, there remains evidence of substantial diurnal variation in biologic onset of ischemic stroke from large-scale observational studies. These findings are reinforced by the presence of a similar morning surge in first detection for ICH and for myocardial infarction, conditions commonly producing pain early after onset that may more often arouse the individual from sleep than ischemic stroke.[10,16]

Diurnal variation in the presenting severity of acute strokes has been probed in several studies. For ischemic stroke, among 1244 patients with stroke in the multicenter FAST-MAG trial (Field Administration of Stroke Therapy - Magnesium), initial clinical deficit severity (National Institutes of Health Stroke Scale) and ischemic core extent (Alberta Stroke Program Early CT Score) were fairly homogenous throughout all day-night time periods.[17] Multimodal imaging studies are warranted to probe for diurnal variation in core, penumbra, collaterals, and infarct growth. For ICH, among 2904 patients in the pooled INTERACT trials (Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage), daytime onset (8 AM to 4 PM) was associated with lesser clinical severity (Glasgow Coma Scale), but no variation in initial hematoma volume or 90-day functional outcomes. However, in a broader registry population of patients with ICH and subarachnoid hemorrhage, 30-day mortality was increased in patients presenting in the morning (6:00 AM to 12 noon).[14] A study in 111 patients with spontaneous ICH found hematoma expansion occurred more frequently in patients presenting in daytime (8 AM to 8 PM).[18]

Given the diurnal variation in vascular physiological parameters, chronopharmacology—drug dosing and discovery taking into account biological rhythm dependencies of agent pharmacological effects and agent pharmacokinetics—is an important consideration in stroke prevention management.[19–21] Nighttime compared with daytime administration of antihypertensives improves overall 24-hour blood pressure profiles.[22,23] Evening compared with upon awakening administration of low-dose aspirin more greatly reduces morning platelet reactivity (which is influenced by the circadian system,[24] via COX-1 [cyclooxygenase 1]-dependent pathways).[25]

Clinical studies of acute stroke treatment and diurnal patterns have focused more upon variations in care delivery throughout the day than upon variations in biologic effect. The effects vary with medical system region. Studies from England, Australia, and from multiple countries in the Safe Implementation of Treatments in Stroke-International Stroke Thrombolysis Register (SITS-ISTR) reported reduced IV thrombolysis rates and longer door-to-needle during night hours and nonworking hours than during working hours.[26–28] In contrast, a large study from Germany reported reduced and slower IV lytic use during working hours than during nonworking hours.[15] In the international SITS-ISTR, after adjustment for patient baseline features, treatment during daytime hours was associated with a small increase in good functional outcome, odds ratio 1.12.[26] Continued investigation of epidemiology and clinical trial databases is warranted to assess the multifactorial effects of circadian rhythms in stroke timing and treatment (Table).

THE NEUROVASCULAR UNIT

Although clinical mechanisms in stroke are complex, the initial response in ischemic tissue is driven by a loss of blood flow that disrupts the supply of oxygen and glucose. Mitochondrial function and ATP regulation all demonstrate circadian rhythm. HIF1 (hypoxia-inducible factor 1), the primary response mediator to hypoxia, interacts with the core circadian genes Clock and Per2.[58] Therefore, at the tissue level, the brain's response to ischemia should be dependent on the time of stroke onset. At the cellular level, emerging literature suggests that circadian biology may affect all cell types in the neurovascular unit.

Table. Circadian Variation in Clinical and Biomarker Features of Human Stroke

	Circadian variation	References
Clinical features		
Risk	Shift work, jet lag, weekday-weekend activity transition increase stroke risk factors	5–9
Onset	Morning surge for ischemic stroke, TIA, and hemorrhagic stroke	10,13,14
	Morning surge for LAA, CE, SV, and CRY ischemic stroke	11,12
Presenting severity	Lesser deficit severity for ICH with daytime onset	14
Physiology	Heart rate, blood pressure, temperature higher in daytime	29–32
	Prothrombotic factors higher in daytime	33–35
	Inflammatory responses increased in daytime	36–53
Outcomes	Increased mortality for ICH and SAH with morning onset	14,17
	More frequent ICH hematoma expansion with daytime onset	18
	More good outcomes for thrombolytic-treated ischemic stroke with daytime presentation	26,28
Chronopharmacology	Antihypertensives more effective with evening administration	22,23
	Low-dose aspirin more effective with evening administration	24,25
Biomarkers		
Melatonin	Night-time levels lower in acute stroke	54,55
Cortisol	Reduced circadian rhythmicity after stroke	54
PAI-1	Early morning peak	35,56,57

CE indicates cardioembolic; CRY, cryptogenic; ICH, intracerebral hemorrhage; LAA, large artery atherosclerosis; PAI-1, plasminogen activator inhibitor; SAH, subarachnoid hemorrhage; SV, small vessel; and TIA, transient ischemic attack.

Neurons are vulnerable to excitotoxicity and oxidative/nitrosative stress, and both pathways are influenced by circadian biology. Diurnal patterns have been described for glutamate receptors and transporters[59] and gamma aminobutyric acid–mediated control of excitability.[60] In rodent models of brain trauma, extracellular glutamate and NMDA receptor levels were dependent on the time of day.[61] In a mouse cardiac arrest model, excitotoxic reductions of hippocampal calbindin were maximal at Zeitgeber time ZT14 (ie, 14 hours after lights-on in animal housing).[62] Similarly, diurnal variations exist for antioxidant genes.[63] Melatonin, a circadian-controlled nocturnal hormone, is a potent antioxidant and potential neuroprotectant,[64] although its effects may be complicated by the fact that it may disrupt diurnal rhythms in glutamate and gamma aminobutyric acid.[65] There is significant crosstalk between circadian genes and enzymes that regulate reactive oxygen species (ROS).[66] Cells deficient in Per2 are more vulnerable to ROS.[67] In neuronal cultures subjected to oxygen-glucose deprivation, glutamate and ROS levels were affected by the stimulation of circadian-like cycles in vitro.[2] In vivo, p53 and Akt (protein kinase B)-regulated neuronal injury after focal cerebral ischemia varied by Zeitgeber time.[68] Knockout of the circadian Bmal1 gene downregulated redox defense and increased oxidative damage.[69] Bmal1 and Per2 may also contribute to the regulation of apoptosis and autophagy. Altogether, these circadian effects on excitotoxicity, oxidative stress, and cell death may be consistent with the observation that Per1 knockout mice were more susceptible to cerebral ischemia.[70] Although detailed molecular mechanisms remain to be dissected, this emerging literature suggests that preclinical neuroprotectant-testing should be adjusted to use active phase models in nocturnal rodents that match active phase human strokes in clinical trials.

Circadian signaling may also influence glia. Extracellular glutamate displays a circadian rhythm that is in-phase with astrocytic calcium.[71] ATP release and ROS buffering capacities in astrocytes were dependent on Bmal1.[72] Consistent with these circadian effects on astrocyte function, neurons co-cultured with Clock-deficient astrocytes become more susceptible to ROS.[73] Circadian effects operate in white matter as well. Microarray analysis of mouse oligodendrocytes and oligodendrocyte precursors demonstrated that genes involved in phospholipid synthesis, myelination, and proliferation were upregulated during the inactive phase, whereas genes involved in apoptosis, stress response, and differentiation were enriched during the active phase.[74]

For stroke, circadian regulation of the vascular compartment should be extremely important. For example, oscillations in resting tone of cerebral arteries display a 24-hour cycle,[75] and vasoactive genes such as eNOS (endothelial nitric oxide synthase) interact with circadian genes.[76] In the penumbra, blood flow may differ during active phase versus inactive phase strokes.[2] Circadian biology affects blood-brain barrier (BBB) function.[77] In Drosophila models, sleep-wake cycles affect BBB permeability[78] and in Bmal1 knockout mice, pericyte coverage of brain microvessels were decreased resulting in leakier barriers.[79] There is also a marked diurnal variation in cerebrospinal fluid production[80] with greater clearance rates during the inactive phase.[81] It has been suggested

that glymphatics[82] and their connections to cervical lymph nodes[83] may contribute to edema, inflammation, and secondary injury after stroke in mice. Therefore, it is possible that circadian biology may influence BBB pathophysiology and edema after reperfusion therapies.

Neurovascular unit mechanisms discussed for ischemia may also be relevant for hemorrhage. Induction of subarachnoid hemorrhage in mice results in higher elevations in Per1 and Per2 during ZT12 compared with ZT2, and this correlates with expression of HO-1 (heme oxygenase 1) and greater reduction in neuronal apoptosis.[84] Conversely, HO-1 knockout mice have reduced expression of clock genes and increased injury, while treatment with carbon monoxide, which is produced by HO-1, restores clock gene expression, and reduces neuronal apoptosis. In mouse models of ICH, sleep-wake patterns are perturbed, and microglial activation is exacerbated.[85] This link between circadian biology and hemorrhage is also documented in humans; Per2 expression in cerebrospinal fluid is higher in patients with ruptured aneurysms compared with controls with unruptured aneurysms.[84]

Circadian biology may also affect stroke recovery. Clock genes are essential for differentiation and fate determination in neural stem cells,[86] and disruption of circadian cycles in mice leads to alterations in hippocampal neurogenesis.[87] In developing zebrafish models, angiogenesis is modulated by Bmal 1 and Per2.[88] Hypoxic regulation of tumor blood vessels shows circadian rhythmicity.[89] Hence, a deeper understanding of how circadian biology influences the remodeling neurovascular unit may help improve the optimization of therapies for stroke recovery and rehabilitation.

Taken together, the emerging literature suggests that circadian rhythms affect the neurovascular unit in ways that influence stroke pathophysiology (Figure). Further studies are warranted to investigate how these mechanisms are regulated and whether these mechanisms may be targeted. Circadian patterns of gene expression may vary in different brain regions depending on age and sex,[90,91] so it remains possible that circadian effects in stroke may depend on lesion location and patient background. Furthermore, there may be feedback loops whereby cortical infarcts indirectly alter the suprachiasmatic nucleus and peripheral clocks. Many gaps in knowledge remain, but ultimately, accounting for circadian biology may assist in the translational effort to defend or repair the neurovascular unit after stroke.

INFLAMMATION AND IMMUNE RESPONSES

The immune system is regulated by circadian biology at various levels. Myeloid cells, such as neutrophils, monocytes, macrophages, as well as lymphoid cells, such as T and B lymphocytes, are known to oscillate in number in blood in both mice and humans.[36,37] Expression of clock genes such as Bmal1 in these cells follows circadian patterns,[38] supporting that this rhythmicity affects the functions of the immune system and its physiological and pathophysiological consequences.

The important role of clock control in myeloid cells is underscored by findings showing that myeloid-specific ablation of the circadian gene Bmal1 leads to a general proinflammatory phenotype in mice, characterized by higher cytokine levels.[39–41] After stroke, mice conditionally Bmal1-deficient in cells expressing CD11b, including microglia, exhibited less potent upregulation of IL6 expression following middle cerebral artery occlusion compared with that in control mice, with a significant attenuation of neuronal damage.[42] This is in agreement with data showing significant reduction in infarct size in female mice after global deletion of Bmal1, in parallel to decreased glial activation.[43] These data together support the important role of circadian regulation of myeloid cell function and its impact on stroke.

In cerebral ischemia, monocytes/macrophages can contribute to both injury and repair.[92] Monocytes infiltrate into brain infarct early after the occlusion[93] and seem to be major players in the prognosis after acute stroke in humans.[94] Importantly, both monocytes and macrophages are known to possess a strong molecular clock,[39,44] and many of the rhythmic transcripts are implicated in crucial innate-immune functions, such as antigen presentation, immune regulation, and phagocytosis.[45] Not surprisingly, monocyte and macrophage molecular clocks are involved in several inflammatory settings in a Bmal1-dependent fashion such as phagocytosis and migration.[37,46,47] Importantly, circadian rhythms are known to regulate macrophage polarization.[37] All these data suggest a possible role of monocyte/macrophage circadian regulation in ischemic brain inflammation.

Closely related to monocytes/macrophages, microglial cells are the first immune responders in the central nervous system. Interestingly, essential microglial functions have been reported to be under the control of an internal molecular clock in physiological[48] and inflammatory conditions,[42,49] an effect in which REV-ERB-α, a nuclear receptor and circadian clock component, may be implicated.[50] Although its specific role in the stroke setting is less known, clock gene disruption in microglia, through the induction of chronic neuroinflammation, has been reported to be involved in the early onset of Alzheimer disease.[51]

Within leukocyte populations, neutrophils play a key role in innate immunity as front-line defensive cells against pathogens and in sterile inflammation. In response to ischemic brain damage, neutrophils are rapidly recruited into lesioned tissue by activated platelets and necrotic cell–derived proinflammatory cytokines and damage associated molecular patterns like HMGB1 or

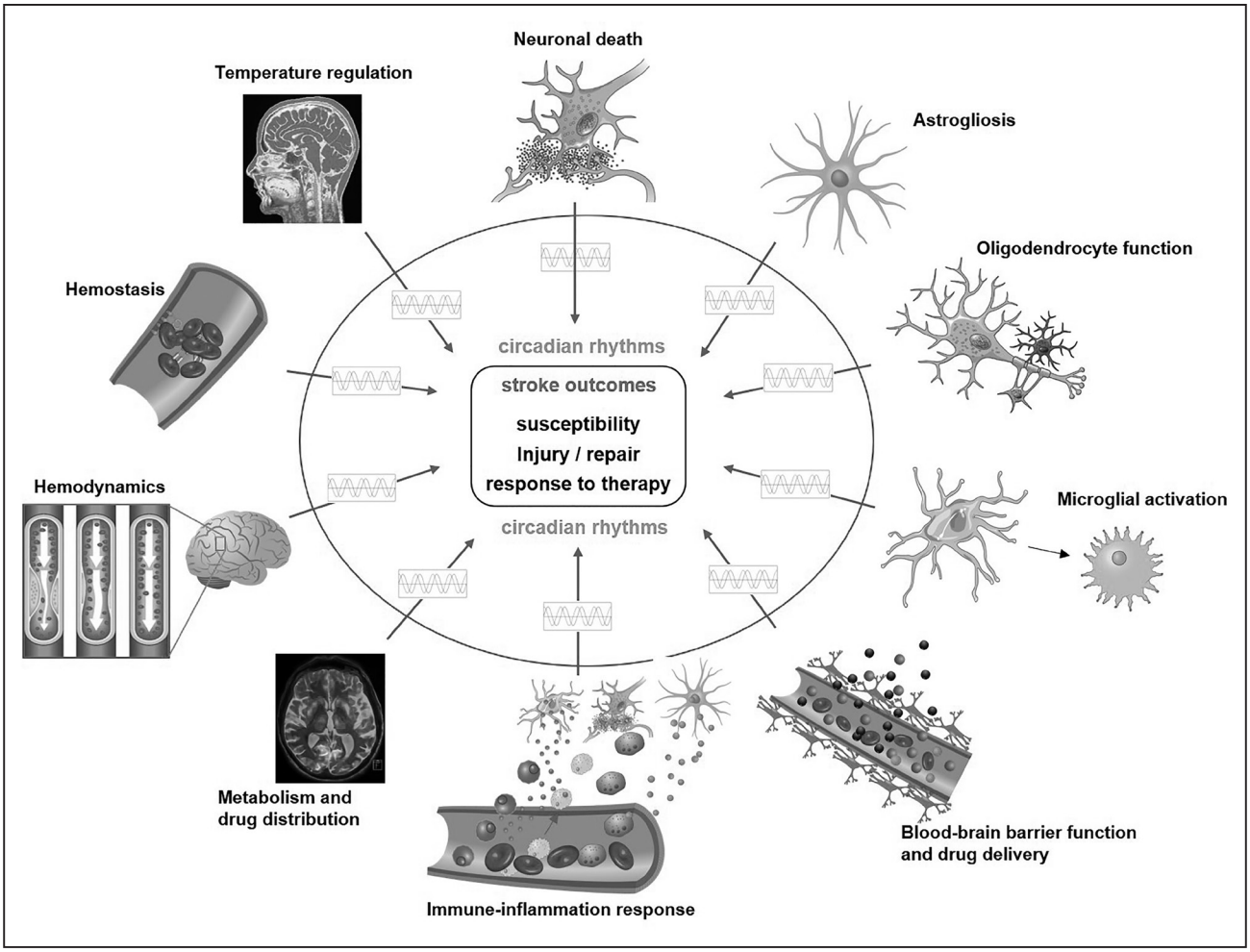

Figure. Circadian rhythms affect multiple molecular, cellular, and physiological pathways that alter susceptibility, injury, and recovery in stroke as well as response to preventative, cerebroprotective, and prorecovery therapies.
The precise circadian timing and coupling of many pathways in brain vs systemic biology are not fully understood. Further investigation into central and peripheral regulation of these various rhythms and pathways in cerebral ischemia and hemorrhage may reveal new approaches for stroke diagnostics and therapeutics.

HSP72 (heat shock protein 72), via the TLR (toll-like receptor) family.[95–98] During this acute stage, neutrophils are instrumental in stroke-associated brain injury, edema, BBB disruption, hemorrhagic transformation,[99–101] and worse neurological outcomes[102] through the release of elastase, production of ROS, and by the no-reflow phenomenon obstructing microvessels.[103] The formation and release of neutrophil-extracellular traps may induce the formation of heterotypic aggregates, thus further contributing to inflammation and thrombosis. However, neutrophils also participate in tissue repair or even have neuroprotective roles, associated with an unexpected heterogeneity of phenotypes for which an internal clock seem to be required. Indeed, circulating neutrophils display circadian oscillations in numbers and phenotype[52] and neutrophil recruitment also oscillates and may influence disease outcome in inflammatory scenarios. Interestingly, whereas in experimental myocardial ischemia, increased cardiac damage during active phase (ZT13) was associated with a greater infiltration of neutrophils in rodents,[104] after myocardial ischemia-reperfusion, lesion sizes were larger in the beginning of light phase, both in mice[53] and in humans,[105] suggesting that circadian differences may be modulated by the presence or absence of reperfusion. Aged CXCR4$^{\Delta N}$ neutrophils aggravate myocardial infarction after ischemia-reperfusion, whereas mice with fresh Arntl$^{\Delta N}$ neutrophils are protected.[53] Strikingly, after permanent occlusion of the middle cerebral artery, brain injury was only exacerbated in mice enriched in fresh neutrophils, suggesting that preferential migration of this subtype during inflammation contributes to tissue injury, although other effects cannot be dismissed.

There may be differences between circadian effects on immune response in heart and brain. Unlike other organs, the brain is devoid of neutrophils at steady state and therefore, parenchymal damage results from infiltrating neutrophils and is unrelated to homeostatic turnover. However, distinct neutrophil phenotypes might also

contribute differentially to the no-reflow phenomenon and/or microthrombosis, mechanisms that remain to be studied. All these findings may be of translational relevance as circadian oscillations are found in human neutrophils.[106,107] In a recent proteomic analysis performed on neutrophils isolated at 8 AM and 2 PM, around 10% of the proteins were differentially enriched between the 2 times in pathways related to vesicle-mediated transport, secretion, exocytosis, or degranulation. Interestingly, ex vivo assays of human neutrophils indicated, for instance, a marked reduction in neutrophil-extracellular trap-forming capacity between 8:00 and 14:00, suggesting key functional differences that may be instrumental for tissue damage after stroke.[107] Altogether, these data suggest that circadian rhythmicity and the molecular clock of immune cells is a major factor in infarct development after stroke (Figure). Future research should ask how to optimize inflammation-targeted therapies for stroke based on time-of-onset.

METABOLISM AND PHYSIOLOGY

In addition to effects at the molecular and cellular levels, circadian biology should also interact with stroke mechanisms involving sleep, hormones, metabolism, temperature and vascular regulation, and drug delivery (Figure). Sleep and circadian rhythm disruption are often present following stroke and poststroke apathy is increased in those with sleep fragmentation and lower sleep efficiency.[108] An important consequence of sleep and circadian rhythm disruption is sustained activation of the stress axis and abnormal release of cortisol.[109] Elevated cortisol over long periods alters metabolism, increases visceral fat, and elevates the risk of type 2 diabetes.[110] Furthermore, cortisol constricts vessels and increases blood pressure, and vascular dysfunction is common in individuals experiencing sleep and circadian rhythm disruption.[111] There may also be bidirectional signaling between circadian control and metabolism. In animal models of diet-induced obesity, local inflammatory responses may disrupt clock genes and shift circadian rhythm in adipose tissue.[112] Ultimately, further investigations are warranted to ask how circadian mechanisms may modulate the effects of physiological comorbidities like hypertension and diabetes in patients with stroke.

One of the most important physiological variables in neuroprotection is temperature, a factor which is regulated by both the circadian system[29] and with sleep-wake cycle[113] with lower temperatures both during the night and during sleep. In patients with stroke, body temperature has a significant influence on infarct size, mortality, and outcome.[114] Hypothermia is well established as an effective neuroprotectant for hypoxia-ischemia,[115] cardiopulmonary bypass surgery,[116] and cardiac arrest[117] but not yet for stroke,[118] although it has been effective in animal models. How circadian biology may modify the neuroprotective and metabolic effects of hypothermia warrants further study.

There are significant interactions between circadian biology and vascular physiology.[30] Elevation of blood pressure in response to exercise appears to be heightened in the morning compared with the afternoon[119] although this may be due to behavioral/environmental factors and not the circadian system, given that under a forced desynchrony protocol, blood pressure reactivity to exercise expresses an endogenous circadian rhythm, peaking in the circadian evening.[31] Others have reported that cerebral autoregulation is also reduced in the early morning,[120] but it is not clear how vascular tone, resistance and vasodilatory responses change upon waking.[119] Further investigation is warranted to test whether circadian regulation of these vascular responses may contribute to differences in stroke incidence and severity throughout the day. More recently, nocturnal hypertension has been proposed as a significant contributor to white matter disease and cognitive decline.[32] Experimental studies suggest that endothelial nitric oxide synthase and nicotinamide-adenine dinucleotide phosphate oxidase, genes implicated in cerebrovascular disease and neurodegeneration, are under the direct control of circadian clocks.[121] Circadian-vascular interactions are also affected by aging as coherence between central and peripheral clocks declines. Hence, it is possible that circadian modulation of vascular and redox function may be involved not only in stroke but also in overall brain health and resilience in the context of an aging cerebrovascular system.

Finally, circadian biology affects drug metabolism and distribution and disruptions in circadian rhythms may affect the response to therapies.[122] For example, sleep/wake cycles in cancer are disrupted, giving rise to the idea that interventions that stabilize circadian systems not only improve quality of life but may also improve survival in patients with cancer. Analogous strategies may apply in stroke, so that the timing of stroke and risk factor medication paired with approaches aimed at stabilizing sleep/wake cycles may present therapeutic opportunities.[4]

BIOMARKERS OF CIRCADIAN RHYTHMS IN STROKE

Circadian biology can be assessed using a wide variety of circulating, imaging, and physiological biomarkers.[123] The majority of circadian studies in stroke have focused on whether stroke disrupts circadian rhythms in circulating molecules and physiological parameters such as blood pressure. Fewer studies have asked whether and how circadian biology modulates pathophysiological processes and treatment effects (Table).

Melatonin is the gold standard to determine the endogenous circadian phase.[123] Two studies found nocturnal circulating and urinary melatonin levels to be lower in patients with acute stroke compared with healthy subjects.[54,55] Patients with poststroke insomnia showed lower circulating levels of melatonin,[124] that is renormalized with light.[125] Cortisol synchronizes 60% of the peripheral circadian transcriptome,[126] and cortisol rhythmicity is often lost after stroke,[54] thus supporting the hypothesis of frequent circadian disruption in patients with stroke. However, whether melatonin or cortisol can identify patients at risk for circadian disruption and monitor circadian disruption poststroke remains to be determined. Future studies may also assess the diagnostic utility of circulating fatty acids,[127] other circulating metabolites,[128] and core clock gene expression in peripheral blood mononuclear cells,[129,130] all known to follow circadian patterns. From a practical perspective, validated circulating biomarkers of circadian rhythms could aid in determining the effect of lesion size and location, and time-of-day of onset on circadian rhythm, and whether circadian disruption relates to delirium and other outcomes after stroke.

Circadian biomarkers may also offer the possibility of optimizing drug delivery. Levels of thrombogenesis[33] and endogenous thrombolysis[34] vary depending on time-of-day of stroke onset. For example, PAI-1 (plasminogen activator inhibitor 1), which is under endogenous circadian control, peaks in the early morning[35] and is regulated by the clock genes PER2, BMAL1, and BMAL2.[56] These variations of hemostasis factors may influence the response to thrombolytic therapies. Patients with recanalized vessels after thrombolysis had lower PAI-1 levels upon admission compared with patients in whom r-tPA (recombinant tissue-type plasminogen activator) did not induce recanalization.[57] However, whether the circadian rhythm of PAI-1 is responsible for the daytime dependence of r-tPA efficacy remains to be explored.

Biomarkers may also assist in studies of circadian rhythms, stroke progression, and outcome. Imaging measures such as penumbra and core volume and circulating levels of neuroaxonal injury markers such as Neurofilament Light Chain[131] would be required to determine whether patients with stroke onset at different times of the day show different progression trajectories of tissue injury. Furthermore, circadian biomarkers might also identify circadian disruptions poststroke. Considering the expected effect sizes, we envision that a large number of patients will be needed to determine such effects in stroke. This information would be particularly relevant for clinical trial recruitment rather than informing on an individual patient's treatment. Ultimately, more research is required to determine whether biomarkers predicting circadian-dependent treatment efficacy may aid in guiding treatment depending on the time-of-day of stroke onset.

TRANSLATIONAL CHALLENGES AND OPPORTUNITIES

This brief review/perspective discussed accumulating evidence suggesting that circadian biology modulates the cerebral response to ischemia and hemorrhage, and these effects may significantly influence clinical mechanisms of susceptibility, injury and recovery in stroke. First, experimental studies have identified circadian variations in many mechanisms that affect stroke pathophysiology, including effects on cell-cell signaling within the neurovascular unit, BBB and glymphatic function, cell death, immune response, as well as neurogenesis and angiogenesis. Second, circadian biology modulates blood flow, hemostasis, metabolism, and temperature regulation, all of which are key variables in stroke. Third, clinical studies from related medical fields such as cardiovascular disease indicate that circadian effects detected in preclinical models may translate to patients, and that the time of disease onset and timing of treatments influences efficacy. A better understanding of how circadian biology influences stroke progression, treatment responses, recovery, and outcome after human stroke could help individualize treatment strategies and improve clinical trial design.

Circadian differences in stroke progression may imply that treatment windows might close faster at certain times, so depending on time-of-onset, some therapies may need to be administered more quickly. Circadian effects on molecular and cellular mechanisms may also mean that some pathways are more or less targetable at certain times. Taking these mechanisms into account may allow one to adapt trial design by defining different time-of-day windows for patient recruitment. This might be particularly relevant in settings when trials are solely based on preclinical studies in nocturnal rodents. Conversely, preclinical testing of stroke therapeutics may also be optimized by adapting the timing of experiments in rodents to match the timing of the majority of diurnal human patients who are recruited into clinical trials. Ultimately, whether clinical stroke progression and treatment responses indeed show diurnal rhythmicity might be investigated with large datasets from prospective observational studies and randomized controlled trials.

Several challenges remain. First, for better translation, we need to improve our understanding of the drivers of diurnal variation in both human and experimental stroke. With similar light input, humans and rodents show only partially overlapping expression patterns of the core clock machinery, have opposing binary activity-rest cycles, and possess different sleep-wake patterns. Specific drivers of diurnal variation such as light-entrained molecular clocks, activity-rest cycles, and homeostatic sleep pressure might differ depending on the targeted mechanism. Second, on a clinical level, it remains to be established how such information could help individualize patient management while taking into account

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the chronotypes (individual circadian timing) of each patient. Third, because clinical studies are confounded by a plethora of factors underlying patient heterogeneity, large patient numbers might be needed to measure circadian variation in a specific end point.

In conclusion, emerging evidence from experimental stroke studies as well as clinical studies in related vascular fields suggest that circadian biology may influence stroke pathophysiology and outcomes. Further research is warranted to expand our understanding of circadian mechanisms in stroke to improve the care and management of patients with stroke.

ARTICLE INFORMATION

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REFERENCES

1. Peirson SN, Foster RG. Bad light stops play. *EMBO Rep*. 2011;12:380. doi: 10.1038/embor.2011.70
2. Esposito E, Li W, E TM, Park JH, Sencan I, Guo S, Shi J, Lan J, Lee J, Hayakawa K, et al. Potential circadian effects on translational failure for neuroprotection. *Nature*. 2020;582:395–398.
3. Thosar SS, Butler MP, Shea SA. Role of the circadian system in cardiovascular disease. *J Clin Invest*. 2018;128:2157–2167. doi: 10.1172/JCI80590
4. Foster RG. Sleep, circadian rhythms and health. *Interface Focus*. 2020;10:20190098. doi: 10.1098/rsfs.2019.0098
5. Chellappa SL, Vujovic N, Williams JS, Scheer FAJL. Impact of circadian disruption on cardiovascular function and disease. *Trends Endocrinol Metab*. 2019;30:767–779. doi: 10.1016/j.tem.2019.07.008
6. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag, chronotype, and cardiometabolic risk. *J Clin Endocrinol Metab*. 2015;100:4612–4620. doi: 10.1210/jc.2015-2923
7. Morris CJ, Purvis TE, Mistretta J, Hu K, Scheer FAJL. Circadian misalignment increases C-reactive protein and blood pressure in chronic shift workers. *J Biol Rhythms*. 2017;32:154–164. doi: 10.1177/0748730417697537
8. Morris CJ, Purvis TE, Hu K, Scheer FA. Circadian misalignment increases cardiovascular disease risk factors in humans. *Proc Natl Acad Sci USA*. 2016;113:E1402–E1411. doi: 10.1073/pnas.1516953113
9. Mason IC, Qian J, Adler GK, Scheer FAJL. Impact of circadian disruption on glucose metabolism: implications for type 2 diabetes. *Diabetologia*. 2020;63:462–472. doi: 10.1007/s00125-019-05059-6
10. Elliott WJ. Circadian variation in the timing of stroke onset: a meta-analysis. *Stroke*. 1998;29:992–996. doi: 10.1161/01.str.29.5.992
11. Ripamonti L, Riva R, Maioli F, Zenesini C, Procaccianti G. Daily variation in the occurrence of different subtypes of stroke. *Stroke Res Treat*. 2017;2017:9091250. doi: 10.1155/2017/9091250
12. Chaturvedi S, Adams HP Jr, Woolson RF. Circadian variation in ischemic stroke subtypes. *Stroke*. 1999;30:1792–1795. doi: 10.1161/01.str.30.9.1792
13. Butt MU, Zakaria M, Hussain HM. Circadian pattern of onset of ischaemic and haemorrhagic strokes, and their relation to sleep/wake cycle. *J Pak Med Assoc*. 2009;59:129–132.
14. Turin TC, Kita Y, Rumana N, Takashima N, Ichikawa M, Sugihara H, Morita Y, Hirose K, Murakami Y, Miura K, et al. Diurnal variation in onset of hemorrhagic stroke is independent of risk factor status: Takashima Stroke Registry. *Neuroepidemiology*. 2010;34:25–33. doi: 10.1159/000255463
15. Reuter B, Sauer T, Gumbinger C, Bruder I, Preussler S, Hacke W, Hennerici MG, Ringleb PA, Kern R, Stock C. Diurnal variation of intravenous thrombolysis rates for acute ischemic stroke and associated quality performance parameters. *Front Neurol*. 2017;8:341.
16. Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med*. 1985;313:1315–1322. doi: 10.1056/NEJM198511213132103
17. Khorramian E, Starkman S, Sanossian N, Liebeskind D, Avila G, Stratton S, Eckstein M, Pratt F, Sharma L, Restrepo L, et al. Marked circadian variation in number and type of hyperacute strokes during the 24 hour day-night cycle. *Stroke* 2020;51. Abstract ATMP71
18. Yao X, Wu B, Xu Y, Siwila-Sackman E, Selim M. Day-night variability of hematoma expansion in patients with spontaneous intracerebral hemorrhage. *J Biol Rhythms*. 2015;30:242–250. doi: 10.1177/0748730415581489
19. Dallmann R, Brown SA, Gachon F. Chronopharmacology: new insights and therapeutic implications. *Annu Rev Pharmacol Toxicol*. 2014;54:339–361. doi: 10.1146/annurev-pharmtox-011613-135923
20. Erkekoglu P, Baydar T. Chronopharmacokinetics of drugs in toxicological aspects: a short review for pharmacy practitioners. *J Res Pharm Pract*. 2012;1:3–9. doi: 10.4103/2279-042X.99670
21. Ohdo S, Koyanagi S, Matsunaga N. Chronopharmacological strategies focused on chrono-drug discovery. *Pharmacol Ther*. 2019;202:72–90. doi: 10.1016/j.pharmthera.2019.05.018
22. Bowles NP, Thosar SS, Herzig MX, Shea SA. Chronotherapy for hypertension. *Curr Hypertens Rep*. 2018;20:97. doi: 10.1007/s11906-018-0897-4
23. Kasiakogias A, Tsioufis C, Thomopoulos C, Andrikou I, Aragiannis D, Dimitriadis K, Tsiachris D, Bilo G, Sideris S, Filis K, et al. Evening versus morning dosing of antihypertensive drugs in hypertensive patients with sleep apnoea: a cross-over study. *J Hypertens*. 2015;33:393–400. doi: 10.1097/HJH.0000000000000371
24. Scheer FA, Michelson AD, Frelinger AL 3rd, Evoniuk H, Kelly EE, McCarthy M, Doamekpor LA, Barnard MR, Shea SA. The human endogenous circadian system causes greatest platelet activation during the biological morning independent of behaviors. *PLoS One*. 2011;6:e24549. doi: 10.1371/journal.pone.0024549
25. Bonten TN, Snoep JD, Assendelft WJ, Zwaginga JJ, Eikenboom J, Huisman MV, Rosendaal FR, van der Bom JG. Time-dependent effects of aspirin on blood pressure and morning platelet reactivity: a randomized cross-over trial. *Hypertension*. 2015;65:743–750. doi: 10.1161/HYPERTENSIONAHA.114.04980
26. Lorenzano S, Ahmed N, Tatlisumak T, Gomis M, Dávalos A, Mikulik R, Sevcik P, Ollikainen J, Wahlgren N, Toni D; SITS Investigators. Within-day and weekly

variations of thrombolysis in acute ischemic stroke: results from safe implementation of treatments in stroke-international stroke thrombolysis register. *Stroke*. 2014;45:176–184. doi: 10.1161/STROKEAHA.113.002133
27. Campbell JT, Bray BD, Hoffman AM, Kavanagh SJ, Rudd AG, Tyrrell PJ. The effect of out of hours presentation with acute stroke on processes of care and outcomes: analysis of data from the stroke improvement national audit programme (sinap). *PLoS One*. 2014;9:e87946.
28. Fang K, Churilov L, Weir L, Dong Q, Davis S, Yan B. Thrombolysis for acute ischemic stroke: do patients treated out of hours have a worse outcome? *J Stroke Cerebrovasc Dis*. 2014;23:427–432. doi: 10.1016/j.jstrokecerebrovasdis.2013.03.029
29. Kräuchi K, Wirz-Justice A. Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol*. 1994;267(3 pt 2):R819–R829. doi: 10.1152/ajpregu.1994.267.3.R819
30. Paschos GK, FitzGerald GA. Circadian clocks and vascular function. *Circ Res*. 2010;106:833–841. doi: 10.1161/CIRCRESAHA.109.211706
31. Scheer FA, Hu K, Evoniuk H, Kelly EE, Malhotra A, Hilton MF, Shea SA. Impact of the human circadian system, exercise, and their interaction on cardiovascular function. *Proc Natl Acad Sci USA*. 2010;107:20541–20546. doi: 10.1073/pnas.1006749107
32. Chesebro AG, Melgarejo JD, Leendertz R, Igwe KC, Lao PJ, Laing KK, Rizvi B, Budge M, Meier IB, Calmon G, et al. White matter hyperintensities mediate the association of nocturnal blood pressure with cognition. *Neurology*. 2020;94:e1803–e1810. doi: 10.1212/WNL.0000000000009316
33. Westgate EJ, Cheng Y, Reilly DF, Price TS, Walisser JA, Bradfield CA, FitzGerald GA. Genetic components of the circadian clock regulate thrombogenesis in vivo. *Circulation*. 2008;117:2087–2095. doi: 10.1161/CIRCULATIONAHA.107.739227
34. Montagnana M, Salvagno GL, Lippi G. Circadian variation within hemostasis: an underrecognized link between biology and disease? *Semin Thromb Hemost*. 2009;35:23–33. doi: 10.1055/s-0029-1214145
35. Scheer FA, Shea SA. Human circadian system causes a morning peak in prothrombotic plasminogen activator inhibitor-1 (PAI-1) independent of the sleep/wake cycle. *Blood*. 2014;123:590–593. doi: 10.1182/blood-2013-07-517060
36. Haus E, Smolensky MH. Biologic rhythms in the immune system. *Chronobiol Int*. 1999;16:581–622. doi: 10.3109/07420529908998730
37. Timmons GA, O'Siorain JR, Kennedy OD, Curtis AM, Early JO. Innate rhythms: clocks at the center of monocyte and macrophage function. *Front Immunol*. 2020;11:1743. doi: 10.3389/fimmu.2020.01743
38. Scheiermann C, Gibbs J, Ince L, Loudon A. Clocking in to immunity. *Nat Rev Immunol*. 2018;18:423–437. doi: 10.1038/s41577-018-0008-4
39. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. *Science*. 2013;341:1483–1488. doi: 10.1126/science.1240636
40. Curtis AM, Fagundes CT, Yang G, Palsson-McDermott EM, Wochal P, McGettrick AF, Foley NH, Early JO, Chen L, Zhang H, et al. Circadian control of innate immunity in macrophages by miR-155 targeting Bmal1. *Proc Natl Acad Sci USA*. 2015;112:7231–7236. doi: 10.1073/pnas.1501327112
41. Sutton CE, Finlay CM, Raverdeau M, Early JO, DeCourcey J, Zaslona Z, O'Neill LAJ, Mills KHG, Curtis AM. Loss of the molecular clock in myeloid cells exacerbates T cell-mediated CNS autoimmune disease. *Nat Commun*. 2017;8:1923. doi: 10.1038/s41467-017-02111-0
42. Nakazato R, Hotta S, Yamada D, Kou M, Nakamura S, Takahata Y, Tei H, Numano R, Hida A, Shimba S, et al. The intrinsic microglial clock system regulates interleukin-6 expression. *Glia*. 2017;65:198–208. doi: 10.1002/glia.23087
43. Lembach A, Stahr A, Ali AAH, Ingenwerth M, von Gall C. Sex-dependent effects of bmal1-deficiency on mouse cerebral cortex infarction in response to photothrombotic stroke. *Int J Mol Sci*. 2018;19:3124.
44. Hayashi M, Shimba S, Tezuka M. Characterization of the molecular clock in mouse peritoneal macrophages. *Biol Pharm Bull*. 2007;30:621–626. doi: 10.1248/bpb.30.621
45. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, Kramer A, Maier B. A circadian clock in macrophages controls inflammatory immune responses. *Proc Natl Acad Sci USA*. 2009;106:21407–21412. doi: 10.1073/pnas.0906361106
46. Carter SJ, Durrington HJ, Gibbs JE, Blaikley J, Loudon AS, Ray DW, Sabroe I. A matter of time: study of circadian clocks and their role in inflammation. *J Leukoc Biol*. 2016;99:549–560. doi: 10.1189/jlb.3RU1015-451R
47. Kitchen GB, Cunningham PS, Poolman TM, Iqbal M, Maidstone R, Baxter M, Bagnall J, Begley N, Saer B, Hussell T, et al. The clock gene Bmal1 inhibits macrophage motility, phagocytosis, and impairs defense against pneumonia. *Proc Natl Acad Sci USA*. 2020;117:1543–1551. doi: 10.1073/pnas.1915932117
48. Hayashi Y, Koyanagi S, Kusunose N, Okada R, Wu Z, Tozaki-Saitoh H, Ukai K, Kohsaka S, Inoue K, Ohdo S, et al. The intrinsic microglial molecular clock controls synaptic strength via the circadian expression of cathepsin S. *Sci Rep*. 2013;3:2744. doi: 10.1038/srep02744
49. Fonken LK, Frank MG, Kitt MM, Barrientos RM, Watkins LR, Maier SF. Microglia inflammatory responses are controlled by an intrinsic circadian clock. *Brain Behav Immun*. 2015;45:171–179. doi: 10.1016/j.bbi.2014.11.009
50. Griffin P, Dimitry JM, Sheehan PW, Lananna BV, Guo C, Robinette ML, Hayes ME, Cedeño MR, Nadarajah CJ, Ezerskiy LA, et al. Circadian clock protein Rev-erbα regulates neuroinflammation. *Proc Natl Acad Sci USA*. 2019;116:5102–5107. doi: 10.1073/pnas.1812405116
51. Ni J, Wu Z, Meng J, Saito T, Saido TC, Qing H, Nakanishi H. An impaired intrinsic microglial clock system induces neuroinflammatory alterations in the early stage of amyloid precursor protein knock-in mouse brain. *J Neuroinflammation*. 2019;16:173. doi: 10.1186/s12974-019-1562-9
52. Casanova-Acebes M, Pitaval C, Weiss LA, Nombela-Arrieta C, Chèvre R, A-González N, Kunisaki Y, Zhang D, van Rooijen N, Silberstein LE, et al. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell*. 2013;153:1025–1035. doi: 10.1016/j.cell.2013.04.040
53. Adrover JM, Del Fresno C, Crainiciuc G, Cuartero MI, Casanova-Acebes M, Weiss LA, Huerga-Encabo H, Silvestre-Roig C, Rossaint J, Cossío I, et al. A neutrophil timer coordinates immune defense and vascular protection. *Immunity*. 2019;51:966–967. doi: 10.1016/j.immuni.2019.11.001
54. Adamczak-Ratajczak A, Kupsz J, Owecki M, Zielonka D, Sowinska A, Checinska-Maciejewska Z, Krauss H, Michalak S, Gibas-Dorna M. Circadian rhythms of melatonin and cortisol in manifest Huntington's disease and in acute cortical ischemic stroke. *J Physiol Pharmacol*. 2017;68:539–546.
55. Ritzenthaler T, Nighoghossian N, Berthiller J, Schott AM, Cho TH, Derex L, Brun J, Trouillas P, Claustrat B. Nocturnal urine melatonin and 6-sulphatoxymelatonin excretion at the acute stage of ischaemic stroke. *J Pineal Res*. 2009;46:349–352. doi: 10.1111/j.1600-079X.2009.00670.x
56. Oishi K, Miyazaki K, Uchida D, Ohkura N, Wakabayashi M, Doi R, Matsuda J, Ishida N. PERIOD2 is a circadian negative regulator of PAI-1 gene expression in mice. *J Mol Cell Cardiol*. 2009;46:545–552. doi: 10.1016/j.yjmcc.2009.01.001
57. Ribo M, Montaner J, Molina CA, Arenillas JF, Santamarina E, Alvarez-Sabín J. Admission fibrinolytic profile predicts clot lysis resistance in stroke patients treated with tissue plasminogen activator. *Thromb Haemost*. 2004;91:1146–1151. doi: 10.1160/TH04-02-0097
58. Kobayashi M, Morinibu A, Koyasu S, Goto Y, Hiraoka M, Harada H. A circadian clock gene, PER2, activates HIF-1 as an effector molecule for recruitment of HIF-1α to promoter regions of its downstream genes. *FEBS J*. 2017;284:3804–3816. doi: 10.1111/febs.14280
59. Chi-Castañeda D, Ortega A. Circadian regulation of glutamate transporters. *Front Endocrinol (Lausanne)*. 2018;9:340. doi: 10.3389/fendo.2018.00340
60. Lang N, Rothkegel H, Reiber H, Hasan A, Sueske E, Tergau F, Ehrenreich H, Wuttke W, Paulus W. Circadian modulation of GABA-mediated cortical inhibition. *Cereb Cortex*. 2011;21:2299–2306. doi: 10.1093/cercor/bhr003
61. Estrada-Rojo F, Morales-Gomez J, Coballase-Urrutia E, Martinez-Vargas M, Navarro L. Diurnal variation of NMDA receptor expression in the rat cerebral cortex is associated with traumatic brain injury damage. *BMC Res Notes*. 2018;11:150. doi: 10.1186/s13104-018-3258-0
62. Tischkau SA, Cohen JA, Stark JT, Gross DR, Bottum KM. Time-of-day affects expression of hippocampal markers for ischemic damage induced by global ischemia. *Exp Neurol*. 2007;208:314–322. doi: 10.1016/j.expneurol.2007.09.003
63. Xu YQ, Zhang D, Jin T, Cai DJ, Wu Q, Lu Y, Liu J, Klaassen CD. Diurnal variation of hepatic antioxidant gene expression in mice. *PLoS One*. 2012;7:e44237. doi: 10.1371/journal.pone.0044237
64. O'Collins VE, Macleod MR, Cox SF, Van Raay L, Aleksoska E, Donnan GA, Howells DW. Preclinical drug evaluation for combination therapy in acute stroke using systematic review, meta-analysis, and subsequent experimental testing. *J Cereb Blood Flow Metab*. 2011;31:962–975. doi: 10.1038/jcbfm.2010.184
65. Marquez de Prado B, Castañeda T, Galindo A, del Arco A, Segovia G, Reiter RJ, Mora F. Melatonin disrupts circadian rhythms of glutamate and gaba in the neostriatum of the awake rat: a microdialysis study. *Journal of Pineal Research*. 2000;29:209-216
66. Putker M, O'Neill JS. Reciprocal control of the circadian clock and cellular Redox State - a critical appraisal. *Mol Cells*. 2016;39:6–19. doi: 10.14348/molcells.2016.2323

67. Magnone MC, Langmesser S, Bezdek AC, Tallone T, Rusconi S, Albrecht U. The Mammalian circadian clock gene per2 modulates cell death in response to oxidative stress. *Front Neurol*. 2014;5:289. doi: 10.3389/fneur.2014.00289
68. Beker MC, Caglayan B, Yalcin E, Caglayan AB, Turkseven S, Gurel B, Kelestemur T, Sertel E, Sahin Z, Kutlu S, et al. Time-of-day dependent neuronal injury after ischemic stroke: implication of circadian clock transcriptional factor Bmal1 and survival kinase AKT. *Mol Neurobiol*. 2018;55:2565–2576. doi: 10.1007/s12035-017-0524-4
69. Musiek ES, Lim MM, Yang G, Bauer AQ, Qi L, Lee Y, Roh JH, Ortiz-Gonzalez X, Dearborn JT, Culver JP, et al. Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *J Clin Invest*. 2013;123:5389–5400. doi: 10.1172/JCI70317
70. Wiebking N, Maronde E, Rami A. Increased neuronal injury in clock gene Per-1 deficient-mice after cerebral ischemia. *Curr Neurovasc Res*. 2013;10:112–125. doi: 10.2174/1567202611310020004
71. Brancaccio M, Patton AP, Chesham JE, Maywood ES, Hastings MH. Astrocytes control circadian timekeeping in the suprachiasmatic nucleus via glutamatergic signaling. *Neuron*. 2017;93:1420.e5–1435.e5. doi: 10.1016/j.neuron.2017.02.030
72. Womac AD, Burkeen JF, Neuendorff N, Earnest DJ, Zoran MJ. Circadian rhythms of extracellular ATP accumulation in suprachiasmatic nucleus cells and cultured astrocytes. *Eur J Neurosci*. 2009;30:869–876. doi: 10.1111/j.1460-9568.2009.06874.x
73. Lananna BV, Nadarajah CJ, Izumo M, Cedeño MR, Xiong DD, Dimitry J, Tso CF, McKee CA, Griffin P, Sheehan PW, et al. Cell-autonomous regulation of atrocyte activation by the circadian clock protein BMAL1. *Cell Rep*. 2018;25:1.e5–9.e5. doi: 10.1016/j.celrep.2018.09.015
74. Bellesi M, Pfister-Genskow M, Maret S, Keles S, Tononi G, Cirelli C. Effects of sleep and wake on oligodendrocytes and their precursors. *J Neurosci*. 2013;33:14288–14300. doi: 10.1523/JNEUROSCI.5102-12.2013
75. Durgan DJ, Crossland RF, Bryan RM Jr. The rat cerebral vasculature exhibits time-of-day-dependent oscillations in circadian clock genes and vascular function that are attenuated following obstructive sleep apnea. *J Cereb Blood Flow Metab*. 2017;37:2806–2819. doi: 10.1177/0271678X16675879
76. Anea CB, Cheng B, Sharma S, Kumar S, Caldwell RW, Yao L, Ali MI, Merloiu AM, Stepp DW, Black SM, et al. Increased superoxide and endothelial NO synthase uncoupling in blood vessels of Bmal1-knockout mice. *Circ Res*. 2012;111:1157–1165. doi: 10.1161/CIRCRESAHA.111.261750
77. Cuddapah VA, Zhang SL, Sehgal A. Regulation of the blood-brain barrier by circadian rhythms and sleep. *Trends Neurosci*. 2019;42:500–510. doi: 10.1016/j.tins.2019.05.001
78. Zhang SL, Yue Z, Arnold DM, Artiushin G, Sehgal A. A circadian clock in the blood-brain barrier regulates xenobiotic efflux. *Cell*. 2018;173:130.e10–139.e10. doi: 10.1016/j.cell.2018.02.017
79. Nakazato R, Kawabe K, Yamada D, Ikeno S, Mieda M, Shimba S, Hinoi E, Yoneda Y, Takarada T. Disruption of Bmal1 impairs blood-brain barrier integrity via pericyte dysfunction. *J Neurosci*. 2017;37:10052–10062. doi: 10.1523/JNEUROSCI.3639-16.2017
80. Nilsson C, Stahlberg F, Thomsen C, Henriksen O, Herning M, Owman C. Circadian variation in human cerebrospinal fluid production measured by magnetic resonance imaging. *Am J Physiol Regul Integr Comp*. 1992;262:R20–R24
81. Hablitz LM, Plá V, Giannetto M, Vinitsky HS, Stæger FF, Metcalfe T, Nguyen R, Benrais A, Nedergaard M. Circadian control of brain glymphatic and lymphatic fluid flow. *Nat Commun*. 2020;11:4411. doi: 10.1038/s41467-020-18115-2
82. Mestre H, Du T, Sweeney AM, Liu G, Samson AJ, Peng W, Mortensen KN, Staeger FF, Bork PAR, Bashford L, et al. Cerebrospinal fluid influx drives acute ischemic tissue swelling. *Science*. 2020;367:eaax7171.
83. Esposito E, Ahn BJ, Shi J, Nakamura Y, Park JH, Mandeville ET, Yu Z, Chan SJ, Desai R, Hayakawa A, et al. Brain-to-cervical lymph node signaling after stroke. *Nat Commun*. 2019;10:5306. doi: 10.1038/s41467-019-13324-w
84. Schallner N, Lieberum JL, Gallo D, LeBlanc RH 3rd, Fuller PM, Hanafy KA, Otterbein LE. Carbon monoxide preserves circadian rhythm to reduce the severity of subarachnoid hemorrhage in mice. *Stroke*. 2017;48:2565–2573. doi: 10.1161/STROKEAHA.116.016165
85. Giordano KR, Denman CR, Dollish HK, Fernandez F, Lifshitz J, Akhter M, Rowe RK. Intracerebral hemorrhage in the mouse altered sleep-wake patterns and activated microglia. *Exp Neurol*. 2020;327:113242. doi: 10.1016/j.expneurol.2020.113242
86. Malik A, Kondratov RV, Jamasbi RJ, Geusz ME. Circadian clock genes are essential for normal adult neurogenesis, differentiation, and fate determination. *PLoS One*. 2015;10:e0139655. doi: 10.1371/journal.pone.0139655
87. Rakai BD, Chrusch MJ, Spanswick SC, Dyck RH, Antle MC. Survival of adult generated hippocampal neurons is altered in circadian arrhythmic mice. *PLoS One*. 2014;9:e99527. doi: 10.1371/journal.pone.0099527
88. Jensen LD, Cao Y. Clock controls angiogenesis. *Cell Cycle*. 2013;12:405–408. doi: 10.4161/cc.23596
89. Koyanagi S, Kuramoto Y, Nakagawa H, Aramaki H, Ohdo S, Soeda S, Shimeno H. A molecular mechanism regulating circadian expression of vascular endothelial growth factor in tumor cells. *Cancer Res*. 2003;63:7277–7283.
90. Abe M, Herzog ED, Yamazaki S, Straume M, Tei H, Sakaki Y, Menaker M, Block GD. Circadian rhythms in isolated brain regions. *J Neurosci*. 2002;22:350–356.
91. Chun LE, Woodruff ER, Morton S, Hinds LR, Spencer RL. Variations in phase and amplitude of rhythmic clock gene expression across prefrontal cortex, hippocampus, amygdala, and hypothalamic paraventricular and suprachiasmatic nuclei of male and female rats. *J Biol Rhythms*. 2015;30:417–436. doi: 10.1177/0748730415598608
92. García-Culebras A, Durán-Laforet V, Peña-Martínez C, Ballesteros I, Pradillo JM, Díaz-Guzmán J, Lizasoain I, Moro MA. Myeloid cells as therapeutic targets in neuroinflammation after stroke: specific roles of neutrophils and neutrophil-platelet interactions. *J Cereb Blood Flow Metab*. 2018;38:2150–2164. doi: 10.1177/0271678X18795789
93. Denes A, Vidyasagar R, Feng J, Narvainen J, McColl BW, Kauppinen RA, Allan SM. Proliferating resident microglia after focal cerebral ischaemia in mice. *J Cereb Blood Flow Metab*. 2007;27:1941–1953. doi: 10.1038/sj.jcbfm.9600495
94. Urra X, Cervera A, Obach V, Climent N, Planas AM, Chamorro A. Monocytes are major players in the prognosis and risk of infection after acute stroke. *Stroke*. 2009;40:1262–1268. doi: 10.1161/STROKEAHA.108.532085
95. Hayakawa K, Pham LD, Katusic ZS, Arai K, Lo EH. Astrocytic high-mobility group box 1 promotes endothelial progenitor cell-mediated neurovascular remodeling during stroke recovery. *Proc Natl Acad Sci USA*. 2012;109:7505–7510. doi: 10.1073/pnas.1121146109
96. Shichita T, Sakaguchi R, Suzuki M, Yoshimura A. Post-ischemic inflammation in the brain. *Front Immunol*. 2012;3:132. doi: 10.3389/fimmu.2012.00132
97. Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, Nácher M, Pitaval C, Radovanovic I, Fukui Y, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science*. 2014;346:1234–1238. doi: 10.1126/science.1256478
98. García-Culebras A, Durán-Laforet V, Peña-Martínez C, Moraga A, Ballesteros I, Cuartero MI, de la Parra J, Palma-Tortosa S, Hidalgo A, Corbí AL, et al. Role of TLR4 (Toll-Like Receptor 4) in N1/N2 neutrophil programming after stroke. *Stroke*. 2019;50:2922–2932. doi: 10.1161/STROKEAHA.119.025085
99. Konsman JP, Drukarch B, Van Dam AM. (Peri)vascular production and action of pro-inflammatory cytokines in brain pathology. *Clin Sci (Lond)*. 2007;112:1–25. doi: 10.1042/CS20060043
100. Buck BH, Liebeskind DS, Saver JL, Bang OY, Yun SW, Starkman S, Ali LK, Kim D, Villablanca JP, Salamon N, et al. Early neutrophilia is associated with volume of ischemic tissue in acute stroke. *Stroke*. 2008;39:355–360. doi: 10.1161/STROKEAHA.107.490128
101. Zhang R, Wu X, Hu W, Zhao L, Zhao S, Zhang J, Chu Z, Xu Y. Neutrophil-to-lymphocyte ratio predicts hemorrhagic transformation in ischemic stroke: a meta-analysis. *Brain Behav*. 2019;9:e01382. doi: 10.1002/brb3.1382
102. Kumar AD, Boehme AK, Siegler JE, Gillette M, Albright KC, Martin-Schild S. Leukocytosis in patients with neurologic deterioration after acute ischemic stroke is associated with poor outcomes. *J Stroke Cerebrovasc Dis*. 2013;22:e111–e117. doi: 10.1016/j.jstrokecerebrovasdis.2012.08.008
103. Ames A 3rd, Wright RL, Kowada M, Thurston JM, Majno G. Cerebral ischemia. II. The no-reflow phenomenon. *Am J Pathol*. 1968;52:437–453.
104. Schloss MJ, Horckmans M, Nitz K, Duchene J, Drechsler M, Bidzhekov K, Scheiermann C, Weber C, Soehnlein O, Steffens S. The time-of-day of myocardial infarction onset affects healing through oscillations in cardiac neutrophil recruitment. *EMBO Mol Med*. 2016;8:937–948. doi: 10.15252/emmm.201506083
105. Suárez-Barrientos A, López-Romero P, Vivas D, Castro-Ferreira F, Núñez-Gil I, Franco E, Ruiz-Mateos B, García-Rubira JC, Fernández-Ortiz A, Macaya C, et al. Circadian variations of infarct size in acute myocardial infarction. *Heart*. 2011;97:970–976. doi: 10.1136/hrt.2010.212621
106. Ella K, Csépányi-Kömi R, Káldi K. Circadian regulation of human peripheral neutrophils. *Brain Behav Immun*. 2016;57:209–221. doi: 10.1016/j.bbi.2016.04.016
107. Adrover JM, Aroca-Crevillén A, Crainiciuc G, Ostos F, Rojas-Vega Y, Rubio-Ponce A, Cilloniz C, Bonzón-Kulichenko E, Calvo E, Rico D, et al.

Programmed 'disarming' of the neutrophil proteome reduces the magnitude of inflammation. *Nat Immunol*. 2020;21:135–144. doi: 10.1038/s41590-019-0571-2

108. Cosin C, Sibon I, Poli M, Allard M, Debruxelles S, Renou P, Rouanet F, Mayo W. Circadian sleep/wake rhythm abnormalities as a risk factor of a post-stroke apathy. *Int J Stroke*. 2015;10:710–715. doi: 10.1111/ijs.12433
109. Phua CS, Jayaram L, Wijeratne T. Relationship between sleep duration and risk factors for stroke. *Front Neurol*. 2017;8:392. doi: 10.3389/fneur.2017.00392
110. Andrews RC, Herlihy O, Livingstone DE, Andrew R, Walker BR. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. *J Clin Endocrinol Metab*. 2002;87:5587–5593. doi: 10.1210/jc.2002-020048
111. Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, Janszky I, Mrkobrada M, Parraga G, Hackam DG. Shift work and vascular events: systematic review and meta-analysis. *BMJ*. 2012;345:e4800. doi: 10.1136/bmj.e4800
112. Prasai MJ, Mughal RS, Wheatcroft SB, Kearney MT, Grant PJ, Scott EM. Diurnal variation in vascular and metabolic function in diet-induced obesity: divergence of insulin resistance and loss of clock rhythm. *Diabetes*. 2013;62:1981–1989. doi: 10.2337/db11-1740
113. Duffy JF, Dijk DJ, Klerman EB, Czeisler CA. Later endogenous circadian temperature nadir relative to an earlier wake time in older people. *Am J Physiol*. 1998;275(5 pt 2):R1478–R1487. doi: 10.1152/ajpregu.1998.275.5.r1478
114. Reith J, Jørgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, Olsen TS. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet*. 1996;347:422–425. doi: 10.1016/s0140-6736(96)90008-2
115. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis. *Arch Pediatr Adolesc Med*. 2012;166:558–566. doi: 10.1001/archpediatrics.2011.1772
116. Engelman R, Baker RA, Likosky DS, Grigore A, Dickinson TA, Shore-Lesserson L, Hammon JW; Society of Thoracic Surgeons; Society of Cardiovascular Anesthesiologists; American Society of ExtraCorporeal Technology. The Society of Thoracic Surgeons, The Society of Cardiovascular Anesthesiologists, and The American Society of ExtraCorporeal Technology: clinical practice guidelines for cardiopulmonary bypass–temperature management during cardiopulmonary bypass. *Ann Thorac Surg*. 2015;100:748–757. doi: 10.1016/j.athoracsur.2015.03.126
117. Nolan JP, Morley PT, Vanden Hoek TL, Hickey RW, Kloeck WG, Billi J, Böttiger BW, Morley PT, Nolan JP, Okada K, et al; International Liaison Committee on Resuscitation. Therapeutic hypothermia after cardiac arrest: an advisory statement by the advanced life support task force of the International Liaison Committee on Resuscitation. *Circulation*. 2003;108:118–121. doi: 10.1161/01.CIR.0000079019.02601.90
118. van der Worp HB, Macleod MR, Bath PMW, Bathula R, Christensen H, Colam B, Cordonnier C, Demotes-Mainard J, Durand-Zaleski I, Gluud C, et al. Therapeutic hypothermia for acute ischaemic stroke. Results of a european multicentre, randomised, phase iii clinical trial. *European Stroke Journal*. 2019;4:254–262.
119. Atkinson G, Jones H, Ainslie PN. Circadian variation in the circulatory responses to exercise: relevance to the morning peaks in strokes and cardiac events. *Eur J Appl Physiol*. 2010;108:15–29. doi: 10.1007/s00421-009-1243-y
120. Ainslie PN, Murrell C, Peebles K, Swart M, Skinner MA, Williams MJ, Taylor RD. Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. *Exp Physiol*. 2007;92:769–777. doi: 10.1113/expphysiol.2006.036814
121. Rodrigo GC, Herbert KE. Regulation of vascular function and blood pressure by circadian variation in redox signalling. *Free Radic Biol Med*. 2018;119:115–120. doi: 10.1016/j.freeradbiomed.2017.10.381
122. Smolensky MH, Hermida RC, Reinberg A, Sackett-Lundeen L, Portaluppi F. Circadian disruption: new clinical perspective of disease pathology and basis for chronotherapeutic intervention. *Chronobiol Int*. 2016;33:1101–1119. doi: 10.1080/07420528.2016.1184678
123. Reid KJ. Assessment of circadian rhythms. *Neurol Clin*. 2019;37:505–526. doi: 10.1016/j.ncl.2019.05.001
124. Zhang W, Li F, Zhang T. Relationship of nocturnal concentrations of melatonin, gamma-aminobutyric acid and total antioxidants in peripheral blood with insomnia after stroke: study protocol for a prospective non-randomized controlled trial. *Neural Regen Res*. 2017;12:1299–1307. doi: 10.4103/1673-5374.213550
125. West AS, Sennels HP, Simonsen SA, Schønsted M, Zielinski AH, Hansen NC, Jennum PJ, Sander B, Wolfram F, Iversen HK. The effects of naturalistic light on diurnal plasma melatonin and serum cortisol levels in stroke patients during admission for rehabilitation: a randomized controlled trial. *Int J Med Sci*. 2019;16:125–134. doi: 10.7150/ijms.28863
126. Reddy AB, Maywood ES, Karp NA, King VM, Inoue Y, Gonzalez FJ, Lilley KS, Kyriacou CP, Hastings MH. Glucocorticoid signaling synchronizes the liver circadian transcriptome. *Hepatology*. 2007;45:1478–1488. doi: 10.1002/hep.21571
127. Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. *Proc Natl Acad Sci USA*. 2012;109:2625–2629. doi: 10.1073/pnas.1114410109
128. Kasukawa T, Sugimoto M, Hida A, Minami Y, Mori M, Honma S, Honma K, Mishima K, Soga T, Ueda HR. Human blood metabolite timetable indicates internal body time. *Proc Natl Acad Sci USA*. 2012;109:15036–15041. doi: 10.1073/pnas.1207768109
129. Braun R, Kath WL, Iwanaszko M, Kula-Eversole E, Abbott SM, Reid KJ, Zee PC, Allada R. Universal method for robust detection of circadian state from gene expression. *Proc Natl Acad Sci USA*. 2018;115:E9247–E9256. doi: 10.1073/pnas.1800314115
130. Wittenbrink N, Ananthasubramaniam B, Münch M, Koller B, Maier B, Weschke C, Bes F, de Zeeuw J, Nowozin C, Wahnschaffe A, et al. High-accuracy determination of internal circadian time from a single blood sample. *J Clin Invest*. 2018;128:3826–3839. doi: 10.1172/JCI120874
131. Tiedt S, Duering M, Barro C, Kaya AG, Boeck J, Bode FJ, Klein M, Dorn F, Gesierich B, Kellert L, et al. Serum neurofilament light: a biomarker of neuroaxonal injury after ischemic stroke. *Neurology*. 2018;91:e1338–e1347. doi: 10.1212/WNL.0000000000006282