CHAPTER

27

Body Clocks in Health and Disease

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INTRODUCTION TO CIRCADIAN CLOCKS

Overview of Circadian Timing

A frustration in the search for mechanisms and treatments for brain-based disorders and diseases is the complex and seemingly overlapping nature of symptoms and causes associated with clinically defined states of health and illness. The circadian timing system, the subject of this chapter, is one of the system level mechanisms that impact an immense array of responses that either antecede or accompany diseases that claim the attention of neuroscientists. These include disturbed sleep/wake cycles and mood regulation, inappropriate food intake and metabolic factors that regulate fat and glucose metabolism and energy expenditure, and physiological responses including stress responses and the accompanying activation of hypothalamic–pituitary axes regulating hormone secretions. A growing body of evidence indicates that circadian "clocks" are located in most cells of the body and that they modulate functions that control emotional, cognitive, and metabolic processes, thereby pointing to mechanisms that can link observed comorbidity in psychiatric and metabolic disruption. Additionally, polymorphisms in genes associated with the cell-based circadian clocks have been associated with sleep disorders and obesity.

Circadian rhythms can best be understood in the context in which they evolved. Living organisms on our planet have internal timekeeping systems, termed circadian "clocks" that enable anticipation of upcoming events for optimal timing of behavior, metabolism, and physiology with respect to predictable changes in the environment. At the level of a cell, system, and whole organism, each function of the body must find its appropriate temporal niche, as it is not possible to do everything all at once. While correlated with daily changes in day and night caused by the rotation of the earth about its axis, circadian rhythms are endogenously organized and are not dependent on timing signals from the environment. That is, even if all time cues are removed, such as occurs in deep underground caves or in outer space, circadian rhythms continue with a period of about 24 h.

One tremendous advantage in the analysis of circadian timing mechanisms is that research

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has advanced to the point that we can monitor time within cells, and/or tissues, or organs or in the behaviors of the whole animal. This is based on our ability to probe the molecular basis of clocks inside cells. The availability of animal models is also a fundamental feature enabling understanding of mechanisms as the molecular elements of cellular clocks are similar in humans and nonhumans, including rodents. Knowledge of molecular circadian clock mechanisms is key to determining the role of clocks in health, dysfunction, and disease.

The aim of this chapter is to provide an overview of the relationships between circadian clocks of the body and the ways in which they can serve to promote health and can cause dysfunction. Some of the hypotheses and research presented in this chapter are derived from very strong experimental evidence, based on the work of many labs, over many years, and confirmed in both animal and human studies. In other cases, we are closer to the frontiers of research and hypotheses and experimental evidence are more tentative. In both cases, we indicate the strength of evidence and direct the reader to relevant in-depth literature reviews.

Defining Circadian Rhythms

Circadian (circa = about, diem = day) rhythms are produced by a biological clock system within the body; they can be distinguished from daily rhythms that are the result of environmental pressures and perturbations. There are several commonly used experimental strategies for distinguishing between endogenously organized and exogenously driven changes (Box 27.1). Furthermore, measures of period, phase, amplitude, and precision are used to assess the status of circadian rhythms. The parameters used for the analysis of circadian periodicity and plasticity include the process of entrainment, determination of period length in free-running conditions, determination of circadian periodicity in response to light disruption (eg, jet lag studies), and evaluation of clock plasticity in non-24-h conditions (termed T-cycles). Studying the properties of circadian (such as their phase, amplitude, and period length) in response to photic perturbation can be particularly useful to help understand how humans respond to jet lag, night shifts, rotating work shifts, or other transient or chronic disruption of environmental surroundings.

Molecular Basis of Circadian Clocks

Circadian rhythms are generated by a clock-like timing mechanism found in virtually all cells of the body. Restated, the fundamental unit of circadian timekeeping is the individual cell. Most generally, the cellular clock is composed of interlocked molecular loops within which the activity of "clock" genes is suppressed by their own protein products. More specifically, circadian clocks in mammals have a highly conserved molecular mechanism based on self-regulating transcriptional–translational feedback loops that underlie rhythmic expression of core "clock" genes (see Fig. 27.1). This consists of the transcriptional activators BMAL1 and CLOCK, which bind to E-box enhancers and activate the transcription of the *Per* and *Cry* repressor gene families. These repressors then feedback to inhibit BMAL1/CLOCK activity, thereby inhibiting their own expression. Each molecular component in the core clock loop is represented by multiple paralogs (eg, *Bmal1*, *Bmal2*; *Per1*, *Per2*, *Per3*; *Cry1*, *Cry2*), which provides the potential for functional redundancy and cell-type specificity. Auxiliary elements such as casein kinase 1/ε (CK1/ε) and glycogen synthase kinase 3, and ubiquitin ligases such as F-box/LRR-repeat protein 3 (FBXL3) fine-tune the period length and amplitude of the molecular circadian clock. One cycle of activation and repression of gene expression takes about 24 h, as befits a daily clock.

BOX 27.1

GLOSSARY OF TERMINOLOGY IN CIRCADIAN RESEARCH

Amplitude. The difference between the peak value and trough value in an oscillation.

Arrhythmia versus Desynchronization. Arrhythmia involves the absence of rhythmicity in a process that is normally rhythmic. Desynchronization refers to the loss of synchrony between a rhythm and its zeitgeber (external desynchronization) or between two rhythms within an organism (internal desynchronization).

Circadian. Circadian events are those having a cycle of about 24 h and include the requirement of being endogenously generated. This is determined by the ability to free-run, with a period of about 24 h, in constant conditions.

Circadian time (CT) versus Zeitgeber time (ZT). Circadian time is a standard unit of time based on the endogenous free-running period of a rhythm. Zeitgeber time is a unit of time based on the period of a zeitgeber, such as the 12:12 light:dark cycle. By convention, in free-running, constant conditions, the onset of activity of day-active organisms is circadian time zero (CT0) and the onset of activity of night-active organisms is CT12. In a light:dark cycle, for diurnal organisms ZT0 (lights on) is the time of activity onset and ZT12 (lights off) defines activity onset for nocturnal animals.

Clock gene. A gene that is an essential element in the cellular–molecular mechanism of circadian rhythmicity.

Entrain/entrainment. The synchronization of a self-sustained endogenous circadian rhythm by a forcing signal (the zeitgeber). In steady entrainment, the period of the self-sustaining oscillation conforms to that of the zeitgeber, and they maintain a stable phase relationship.

Food- versus light-entrainable oscillator. The SCN is a light-entrainable oscillator. A food-entrainable oscillator is a putative circadian pacemaker, locus unknown, that can be entrained by regularly scheduled food restriction but not by a light–dark cycle.

Free-run/free-running. A self-sustaining oscillation or rhythm that is retained in the absence of effective zeitgebers or other environmental agents that may affect the period of the oscillation.

Masking. Disruption or elimination of the expression of an overt rhythm caused by an external agent, without a direct effect on the period or phase of a pacemaker.

Pacemaker. An entity that can generate endogenous rhythmicity and impose this rhythmicity on other entities. The SCN is a pacemaker. Note that while a pacemaker is an oscillator, not all oscillators are pacemakers.

Phase/phase angle/phase shift. The relative angular displacement between a periodic quantity and a reference angle. A phase shift is a displacement (in time) of an oscillation.

Precision. The daily stability of the clock over successive days. Under free-running conditions, a highly precise clock will time the onset of behavioral activity or physiological processes to the same time of day each day, while a less-precise clock may encounter drift from day-to-day, sometimes starting early, other times starting late.

Subjective day/subjective night. In free-running, constant photic conditions (where day and night are not signaled by light–dark cycles), the segment of a circadian cycle that corresponds to the day is the subjective day (active time for the diurnal animal and inactive time for the nocturnal animal). The segment of a circadian cycle that corresponds to the night is the subjective night (inactive time for the diurnal animal and active time for the nocturnal animal).

Zeitgeber/zeitgeber time. A synchronizing signal capable of resetting a pacemaker or synchronizing a self-sustaining oscillation.

When core clock genes are mutated, the consequence is altered period, amplitude, or robustness of circadian cycles. These can be tracked in behavioral measures of locomotor activity and sleep–wake patterns in animals or humans, or in altered rhythms of electrical activity or clock gene expression in SCN cells, or in tissues as a whole, in vivo or in vitro. Mutations of auxiliary elements that modulate the core clock genes also affect these parameters. For example, hypophosphorylation of *Per2* because of a mutation of the phospho-acceptor site of human PER2 protein, seen in subjects suffering from familial advanced sleep phase syndrome (FASPS), shortens circadian period by 2–4 h. Early nighttime sleep onset is accompanied by early occurrence of many responses including sleep onset and wakening, minimum core body temperature, and evening onset of melatonin secretion.

Clock-Controlled Genes

Circadian clocks have a broad impact throughout the body, because downstream genes are rhythmically activated by core clock genes. More specifically, in addition to the core clock genes such as *Per and Cry*, the CLOCK/BMAL complex also drives rhythmic expression of numerous output genes, termed clock-controlled genes. Examples include the control of metabolism by rhythmic nuclear receptors, the modulation of the immune response, and diurnal rhythms of detoxification via *Alas1*. The clock-controlled genes encode proteins that are involved in tissue-specific regulatory and metabolic activities. Genome-wide transcription analysis suggests that clock-controlled genes have only about 10% overlap from tissue to tissue, and have different peak phase distributions in various organs. Circadian influences have been widely documented and include the immune and metabolic systems, sleep–awake cycles, memory consolidation, blood pressure, body temperature, cell division and proliferation, hormone secretion, apoptosis, and senescence. Tissue-specific differences in rhythm generation may be attributable to variations of the core clock genes across tissues, tissue-specific rhythmicity in transcription factors and co-factors, or sensitivity to systemic cues originating in hormone secretions, immune system responses, sympathetic innervation, body temperature, and activity rhythms. Understanding the complexity of tissue-specific circadian expression patterns and how to use them to optimize administration of therapeutic drugs is currently a major research area.

The Master Clock Lies in the Suprachiasmatic Nucleus

There is substantial evidence that a "master" clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, guides the temporal structure of a hierarchical system of molecular–cellular clocks located throughout the body, acting to set the phase of rhythms throughout the body (Fig. 27.1). The SCN lies above the optic chiasm, and has about 10,000 cells on each side of the third ventricle (in rodents). When these cells are ablated, animals lose circadian rhythmicity, and never recover. However, rhythmic activity/rest cycles can be restored by brain tissue transplants containing the SCN, and the genotype of the grafted SCN determines the period of the restored rhythm. Thus, if the donor tissue has a period of 20 h, while the host originally had a period of 24 h, the restored rhythm is strictly that of the donor. The lesion and transplant data together constitute strong evidence that the SCN is the sole origin of the circadian signal that supports circadian oscillation and determines the phase of the behavioral and physiological responses.

Output Signals of the Suprachiasmatic Nucleus

The SCN master clock, directly or indirectly, is the source of signals that synchronize peripheral

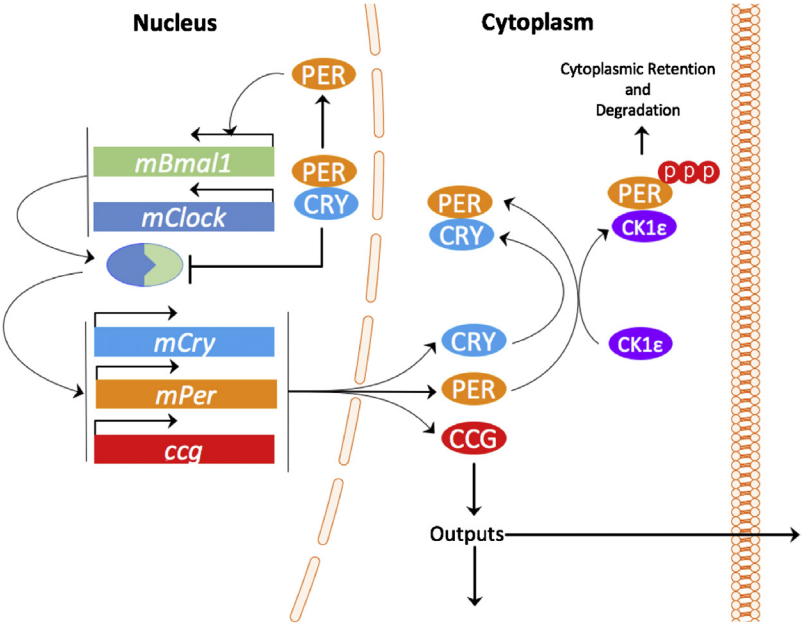

FIGURE 27.1 A simplified illustration of the core circadian clock mechanism depicting the transcriptional–translational feedback loop in mammals. At the core of this loop there is, in the nucleus, a BMAL1/CLOCK protein complex that binds to the promoters of the *Per* and *Cry* family of genes, as well as to clock-controlled genes (*ccg*). This results in increased transcriptional activity of these targets. In the cytoplasm, the PER/CRY proteins dimerize and translocate back to the nucleus to inhibit BMAL1/CLOCK and block their own transcription. In parallel, kinases such as casein kinase 1 epsilon (CK1e) phosphorylate PER protein in the cytosol, targeting them for degradation. This provides additional time delays enabling the molecular clock to cycle with a period of about 24 h.

clocks which themselves contain oscillators. This yields a system in which some aspects of mammalian physiology are controlled directly by central signals and others by peripheral clocks. The SCN can signal the rest of the body by many different routes. First, there are monosynaptic and multisynaptic projections that reach the central and peripheral nervous systems. Secondly there is an important role for peptidergic and hormonal signals that circulate locally or via systemic circulation. Finally, the SCN also acts indirectly, via behavioral effects on food intake and temperature, to entrain peripheral clocks. The SCN and peripheral clocks respond to different signals. For example, the SCN does not respond to food-related or thermal cues. Glucocorticoids of the adrenal gland are secreted rhythmically (orchestrated by the SCN), and their receptors are expressed in most peripheral tissues, but not in SCN neurons. Furthermore, in vitro studies indicate a myriad of other pathways, involving signaling by kinases and calcium that can shift peripheral clocks.

Extra-Suprachiasmatic Nucleus Clocks in Central Nervous System and Periphery

The discovery of clock genes in extra-SCN brain regions and in the periphery is of crucial importance in understanding the importance of temporal order in both health and disease (Fig. 27.2). Clocks have been found in many brain regions, including the prefrontal cortex, hippocampus, and amygdala, where they are thought

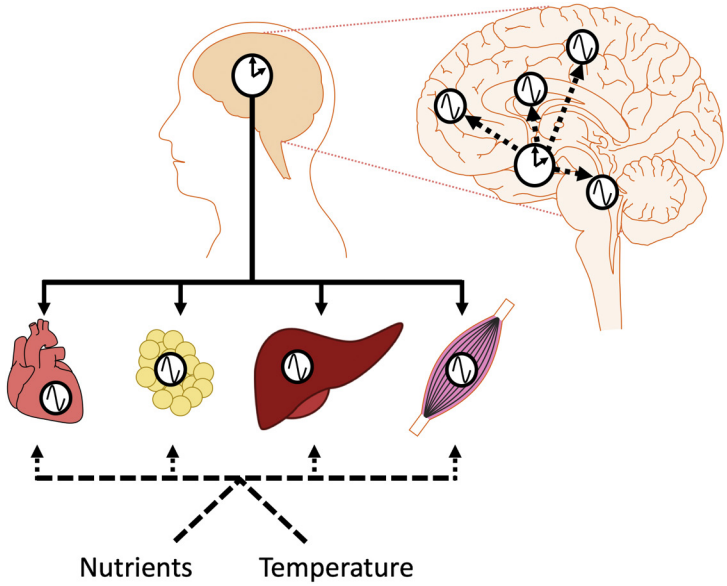

FIGURE 27.2 The master circadian clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The phase of the SCN is adjusted to geophysical time every day by light signals detected in the retina. The phase of clocks in extra-SCN brain regions (such as the cortex, hippocampus, and amygdala) are set by both neural and diffusible signals from the SCN. Clocks in peripheral tissues, such as the heart, adipose, liver, and skeletal muscles, are synchronized by the SCN through a variety of systemic signals, including metabolites, cytokines, body temperature, and peripheral nervous system signals. In addition, nutrients from food, as well as changes in body temperature, can help to synchronize these peripheral clocks. In most tissues, the SCN is necessary for synchronization of individual cellular oscillators, though in some tissues (eg, olfactory bulbs) individual oscillators remain coupled even in the absence of systemic signals from the SCN (see text for further details).

to help regulate homeostatic, cognitive, and emotional function. Rhythmic gene expression has also been found in several hypothalamic nuclei, including the bed nucleus, paraventricular nucleus, and arcuate nucleus. These rhythms are thought to influence numerous homeostatic functions from feeding to reproduction. Rhythmic gene expression is also found in the reward circuitry, with known effects on diverse motivated behaviors. A similar pattern is observed in peripheral organs, including the heart, adipose tissue, liver, and skeletal muscle, where rhythmic clock gene expression is clearly observed. The mechanisms by which these local clocks modulate downstream function still remain a mystery, but current data show that they modulate a vast number of genes. Therefore, their dysregulation in the brain and peripheral tissues is thought to provoke a wide range of pathologies. The relatively familiar temporal dysregulations evident in jet lag and shift work, are produced by differential rates of adjustment of cellular clock and their downstream targets in different tissues. Pathologies such as metabolic syndrome, immune dysfunction, and cancer are additional conditions that accompany and perhaps result from conflict between peripheral and central clocks. While true that nearly all extra-SCN oscillators dampen following upon the removal of the synchronizing influence of the SCN, the olfactory bulb shows sustained rhythmicity that is independent of the SCN. Thus there exist both SCN-dependent and SCN-independent oscillators.

Much evidence supports the hypothesis that cellular physiology is tightly regulated in time, measured by regularly recurrent daily oscillations of gene expression in a tissue-specific metabolic program. Tissue-specific oscillators have unique molecular components, so that the synchronizing factors to which they respond, and their cellular outputs, can be regulated locally. As discussed later, these local tissue–specific molecular factors, especially in extra-SCN regions of the brain, are not well understood.

Suprachiasmatic Nucleus Clocks Differ From Other Tissue Clocks

Insight into how the cells of the brain and body subserve the functions necessary for healthy survival can be understood in the beautifully plastic hierarchical organization of the circadian timing system. As noted, the SCN does not respond to temperature fluctuations. Thus, inverting environmental day–night temperature changes will inverse circadian gene expression in peripheral cells and non-SCN brain regions, but not in the SCN itself. The insensitivity to temperature changes disappears in dispersed SCN cells, indicating that it is a property of its network organization and not attributable to its individual cells. Its network properties enable the SCN to continue to oscillate for many months in vitro, again proving that this is an intrinsic oscillation—not requiring any external input. Interestingly, circadian clock genes and proteins harvested from neural and nonneural tissues, including liver, lung, kidney, SCN, and some brain regions outside the SCN, also continue to oscillate when they are placed in culture. However, in non-SCN tissues, these oscillations tend to damp after several days, likely because their individual cells eventually go out of phase with each other. The circadian oscillations can be resynchronized and thus restarted for another series of days by chemical signals such as those produced by changing the culture medium.

These findings evoke the question of how clock cells of the SCN differ from those in the rest of the body. Part of the answer lies in the complement of clock genes and in the nature of communications among cells in various tissues. While "core clock genes" are present in most tissues, the homologous genes can assume different tissue-specific functions. For example, there is a functional overlap between CLOCK and its homolog NPAS2. However, knockout of CLOCK protein is compensated by the presence of NPAS2 in the SCN, and *Clock* mutant mice are behaviorally rhythmic. In contrast, the deletion of CLOCK produces arrythmicity of circadian oscillators in peripheral tissue explants.

A unique aspect of the SCN is not only the network organization of its neurons but also their access to signals from the external and internal environment. Mammalian tissue clocks, unlike those of other species such as flies, are not intrinsically light sensitive and so, in vivo, depend completely on the SCN for entrainment to the solar day. In mammals, photic input from the retina travels along the retinohypothalamic tract and reaches a subset of the oscillating SCN clock cells via a monosynaptic connection. These directly retinorecipient neurons synchronize the SCN to the external solar day and the SCN then communicates temporal information to peripheral clocks via endocrine and systemic cues. Importantly, the same cells that are directly retinorecipient also are sensitive to temporal information derived from internal cues provided from other parts of the body. As an example, retinorecipient SCN cells in males also contain androgen receptors. Removing the endogenous source of androgens (from testes), significantly alters the function of the SCN, and causes a lengthening of the circadian period measured by behavioral locomotor activity. These are direct, local effects of androgens on the SCN, and do not require actions at other neural sites or on muscle. This finding is consistent with several studies demonstrating that the concentration of androgens reaching the SCN via the general

circulation modulates the period of activity rhythms (eg, the higher the concentration of androgens, the shorter the circadian period). Remarkably, both the pattern of androgen receptor expression and androgenic effects on circadian behavior show a clear sexual dimorphism, and this may be key to understanding changes in period and sleep onset times that occur with age (see section: Temporal Order and Phase-Setting Signals in a Life Span Perspective). It is not only gonadal hormones that are peripheral signals that can alter clock function, but also feeding-related and metabolic signals also have very strong effects on circadian timing as well, and are discussed later in the context of obesity and metabolic disease.

Light Sets Suprachiasmatic Nucleus Phase

Because light from the daily day–night cycle is the most salient phase-setting cue, the retinal input to the SCN is considered one of its most important unique features. The endogenous rhythm of the SCN is about 24 h; in humans it tends to be somewhat longer than 24 h, while in mice it tends to be somewhat shorter. To remain synchronized to the daily day–night cycle, the SCN must be reset each day. To do this, the SCN clock shifts its phase in response to light cues during its dark phase. Information about light in the environment reaches the SCN through specialized neurons in the eye called intrinsically photosensitive retinal ganglion cells. In mammals, this is the only system whereby photic information reaches the brain.

Photic Versus Visual Afferents to the Central Nervous System

The intrinsically photosensitive retinal ganglion cells serve the SCN, but do not support vision. Even in mice that are completely blind visually (due to the lack of rods and cones), information from intrinsically photosensitive retinal ganglion cells can synchronize the SCN, and in the absence of these cells photic cues detected by melanopsin or classical photo pigments cannot reach the SCN. Specifically, genetic elimination of melanopsin-containing ganglion cells completely abolishes the ability of mice to set their brain clock. On the other hand, these mice possess normal visual abilities, proving that visual function and resetting the phase of the brain's clock constitute two separate afferent systems. Similar mechanisms occur in people: If the retinal input to the SCN is intact, visually blind individuals can continue to synchronize to the daily light:dark cycle, though they may be totally unaware of the presence or absence of light in their environment. Thus, by virtue of its network of interconnected oscillators, its ability in situ to both synchronize to photic cues of the external environment and to generate endogenous signals within the body is what differentiates the SCN clock from oscillators in other tissues.

COHERENT VERSUS DISRUPTED TEMPORAL ORDER

In modern industrialized societies, the advent of electric lighting has added substantial environmental perturbation to the circadian timing system. In many ways, today our internal clocks are constantly under pressure from changes in light cycles, altered activity patterns, and new social norms. The American Medical Association, in recognition of serious adverse consequences, has adopted a policy statement on the adverse health effects of nighttime lighting, acknowledging a direct link between disrupted circadian rhythms and cell cycle regulation, DNA-damage responses, and metabolism. In recognition of the evidence that nurses who work at night have an increased risk of developing breast cancer, the Danish government, since 2009, pays compensation to women who developed breast cancer after working at night. The mechanisms whereby such environmental

disruption produces vast effects on the body can be understood in the context of circadian temporal organization.

Temporal Order and Phase-Setting Signals in a Life Span Perspective

The temporal order of the body is normally synchronized such that sleep, wake, feeding, and other functions are anticipated, occur at appropriate times, and do not interfere with each other. To understand optimal temporal structure, it is important to take a life span perspective. A systematic change in circadian rhythms occurs over a lifetime, measured by changes in rhythm precision, amplitude, and phase. New born infants are nearly arrhythmic, with irregularly occurring episodes of sleep and wakefulness over a day. Over the course of many months, there is a consolidation of sleep–wake cycles, and less prominent circadian responses such as temperature and heart rate. The timing of sleep onset, for example, is progressively later from early childhood to young adulthood, and then becomes gradually earlier into old age. Along with sleep, there are changes with age in the amplitude of hormone secretion (eg, melatonin), body temperature, and other physiological parameters. These age-related phase changes of our circadian rhythms can have severe practical social consequences.

People can be characterized as having different sleep types ("chronotypes") ranging from very early active "larks" to very late active "owls." Extreme larks and owls may not even overlap in their sleep–wake cycles. Furthermore, on average, the chronotype changes significantly during the lifetime of an individual, and timing of sleep is markedly affected by the schedule requirements of work and school. On average mid-sleep time for children, on nonschool days, is about 3:30 am and by early adulthood it is about 5 am. Mid-sleep time then gradually advances to earlier than 3 am in 75–80 year olds. If we assume that the average sleep duration is about 8 h, this means that the normal wake-up time is roughly 7:30 am for elementary school children and 9 am for college students. For these young people, the phase of the internal biological clock is not well adjusted to the socially imposed phase. This has given rise to the idea that in these conditions, young people suffer from "social jet lag," which appears to contribute to poorer performance, and possibly to obesity.

Many of us become familiar with the temporal order of the body only when it is disrupted, as occurs during jet lag. The problems associated with jet lag as a result of travel to a new time zone and changes in the environmental light–dark cycle provide clues to the importance of temporal structure. The disruptions consequent to jet lag are well understood. As we know, human sleep occurs almost entirely at night, during the dark, and not surprisingly, light is a major factor in setting the timing of human sleep. Light at night resets the phase of circadian rhythms, but the ability of the SCN clock to adjust to daily photic resetting signals is limited. In humans tested under controlled laboratory conditions, the circadian clock can maximally advance its phase by about 2 h/day and can delay its phase by about 3 h/day under optimal conditions. That is why it takes several days to adjust to a new time zone after long-distance travel. During the time of adjustment, we suffer from jet lag, since our internal timing system is in conflict with geophysical time. This manifests itself by perturbed sleep–wake cycles, indigestion problems, urination during the night, fatigue, and disrupted cognitive function.

Sleep–Wake Cycles

The rhythm most obvious to us is the daily alternation between wake and sleep. An intuitively pleasing conceptualization of the circadian contribution to the timing of sleep was captured decades ago in the two-process model of sleep—one of the most widely examined hypotheses

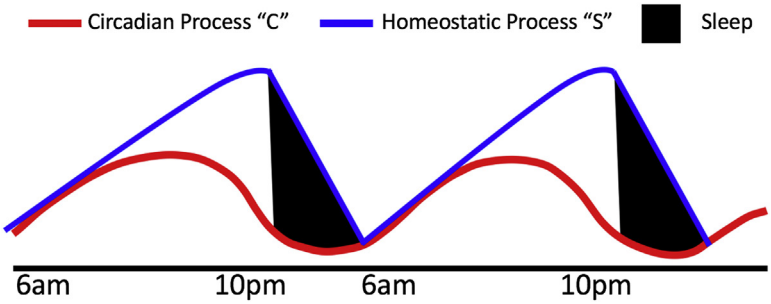

FIGURE 27.3 The two-process model of sleep posits that time spent awake drives a homeostatic need for sleep (Process "S"), represented at the physiological level by buildup of sleep-promoting substances such as adenosine among others. In parallel, the circadian drive (Process "C") promotes wake during what is supposed to be the active phase (eg, daytime in humans), which rhythmically wanes later in the evening. When sleep pressure is high (peak of Process "S") and circadian drive is low (trough of Process "C"), sleep is initiated. Sleep pressure is dissipated through the night, and the circadian drive for wake begins its rhythmic increase early in the morning. This interactive two-process system ensures that sleep need and sleep timing are properly balanced.

regarding sleep regulation. The key element of this model is the proposed interaction of two constituent processes—the sleep–wake homeostatic process, termed "S" and the circadian process, termed "C," to generate the timing of sleep and waking (Fig. 27.3). Process "S" is driven by the buildup of sleep-inducing substances that increase while you are awake. Once you begin to sleep, the levels of these substances decline throughout the sleep period, and will begin to build up once again upon awakening. Potential sleep substances are many, and they include adenosine, some cytokines, and likely many as-yet-unknown compounds. Process "C" on the other hand is driven by the circadian clock, and SCN signals act to stimulate waking. Thus, under normal conditions, wake drive is lowest in the late evening, when process "S" sleep drive is reaching a peak. It is at this point that sleep is induced—at the intersect of these two processes. As sleep need diminishes, and the circadian drive for wake increases once again in the early morning, the sleep bout is terminated and awakening follows. Restated, if you stay awake for 24 h, then as time progresses, you start to feel alert again as the circadian input signals alertness, even though sleep pressure–derived homeostatic process S continues to increase.

Of course, many additional external factors can influence sleep, including work requirements, alarm clocks, ambient temperature, naps, stress, exercise, meal times, and types of food eaten. There is some evidence that foods containing tryptophan (bananas, milk) or carbohydrates (bread) promote sleep, while those containing tyramine (ham, avocado, nuts, red wine) promote night time wakefulness. Also, psychoactive drugs can act either as sedatives or as stimulants to enhance or reduce sleep and advance or delay sleep onset. A multitude of effects modulate circadian timing of sleep, and several industries have arisen to advocate for "sleep hygiene." In the following sections, we explore some of the consequences associated with disruption of temporal order.

METABOLISM AND OBESITY: PERIPHERAL CLOCKS CONTRIBUTE TO METABOLIC DISORDERS

Mechanistic Links Between Circadian and Metabolic Processes

Environmental perturbations can be disruptive, leading to a search for the mechanisms

by which they perturb circadian timing and thereby impact metabolic processes. The search for potential mechanisms at the molecular level entails examination of the genes and molecules that provide links between the circadian clock and metabolic processes. It is very clear that the circadian system and metabolic systems are intimately linked. Disruption of the circadian clock hierarchy is observed in obesity and metabolic syndrome, but it is yet to be established whether disruption of the circadian clock is a *symptom*, or a *cause*, of metabolic dysregulation. One way to probe the causal connection between circadian disruption and metabolic disorders is to disrupt clock genes and observe the effects on metabolic function. A significant finding in this regard was a series of studies in which whole-body *Clock* mutant mice were used to probe changes in metabolism. In *Clock* mice, the circadian clock is altered from birth in both central and peripheral tissues. These mice show marked development of obesity, which is a result of decreased metabolic rate and reorganization of feeding patterns. That is, *Clock* mice eat more during the light period than do WT mice. This whole animal model clearly shows that genetic disruption of clock function in the whole animal can lead to metabolic dysregulation. However, it does not provide adequate resolution to determine the precise mechanisms by which this occurs.

Because obesity and metabolic syndrome are associated with defects in glucose homeostasis, this is a logical area of investigation to investigate effects of mutations in circadian clock genes. The first question becomes: how are glucose and insulin altered by genetic mutations of the clock? In humans, polymorphisms in both *Clock* and *Bmal1* have been implicated in the development of Type II diabetes. In animal studies, whole body mutants of *Clock* and *Bmal1* demonstrate impaired glucose tolerance and blunted insulin insensitivity, and mice with mutated *Per2* show increased plasma insulin as well as impaired liver gluconeogenesis. Similarly, knocking out both *Cry1* and *Cry2* causes a hyperglycemic response to acute feeding and impaired glucose tolerance.

How can altering clock gene expression lead to such marked changes in systems controlling these complex metabolic processes? If the molecular clocks are disrupted throughout the body, the central and peripheral clocks no longer function in concert, and this temporal disorganization contributes to metabolic dysregulation, weight gain, and eventually obesity. This can be understood by considering CCG's. Peripheral tissues show rhythms not only in the canonical "clock" genes, but also in thousands of other genes governing nearly all cellular processes. It has been estimated that between 10 and 20% of genes in the liver are rhythmic, and about 15–20% of hepatic proteins show circadian rhythms. Modern bioinformatics techniques, linking gene transcript levels to protein levels and metabolic outputs of biochemical pathways have revealed the intricate temporal relationship in the liver. Taken together, it appears that clock function in peripheral organs is very important for overall function of these tissues.

Today, tools are available for probing the role of specific peripheral clocks using genetic targeting techniques that can disrupt clock genes in specific organs, including some essential for normal glucose homeostasis. Using this clever approach, the specific role of circadian clock genes in particular organs can be interrogated. As an example, it is now known that genetically knocking out the core clock genes *Clock* and *Bmal1* specifically in pancreatic beta cells (leaving them unaltered in other tissues) results in a diabetic phenotype, including very high levels of plasma insulin, and impaired glucose homeostasis.

Circadian Rhythms and Metabolic Function: A Two-Way Relationship

On the one hand, disrupted circadian clocks contribute to metabolic disorder, but it is also the case that this is a "two-way street," and

metabolic factors—particularly those involved in the development of obesity and/or diabetes—can also modulate circadian and sleep systems. As an example, if food is presented to a nocturnal animal during the day, the timing of oscillations in peripheral clocks will be inverted, though the clock gene oscillations in the SCN are not affected. Another example, drawn from genetic mutant animals, the *Clock* double mutant mouse can entrain to food, even when its circadian locomotor behavior in the absence of food cues is arrhythmic. The quality of nutrition can also change eating behavior and affect the peripheral clock. A high-fat diet produces obesity and a damped feeding rhythm in mice so that they eat more food during the day and similarly reduces rhythmic gene expression in the liver. Furthermore, when food is withheld for 24 h, the number of oscillating hepatic transcripts is reduced to only a few hundred transcripts while controls who consume the same amount of food as ad libitum-fed counterparts show robust rhythm in 5000 transcripts. Importantly, high-fat diet-induced obesity can be prevented by maintaining a strict feeding–fasting rhythm without reducing caloric intake. Similarly, the timing of food availability can "rescue" arrhythmic phenotypes in genetic clock mutants. For instance, Cry1/Cry2 double mutant mice, which completely lack a functional circadian clock, have erratic eating patterns, and lack rhythms in the liver. However, when these genetically modified mice are subject to a feeding–fasting regimen, several hepatic transcripts and metabolites assume daily rhythms characteristic of normal temporal structure.

The evidence is strong that the master circadian clock in the SCN can be entrained by light, while the phase of oscillators of other tissues, such as the liver, can be reset by cues derived from the timing of food intake. However, while the effects of food-derived signals are very well established, the mechanism(s) of entrainment by food is unclear. That said, the independent effects of light and food on SCN and peripheral clocks are likely one mechanism whereby poor temporal structure can arise. Circadian rhythm research in peripheral organs has revealed the importance of eating patterns on peripheral gene expression patterns, uncovered genetic and nutrition factors that modulate eating pattern, and demonstrated that eating patterns can counteract some obesogenic effects.

In humans and nonhuman animals, obesity is linked to disrupted sleep and circadian rhythms. This is likely due to a variety of factors, including clinical disorders such as sleep-disordered breathing, which can lead to sleep disruption and are more prevalent in obese or overweight individuals. Animal studies also indicate that obesity can lead to altered circadian and sleep parameters, including a change in the amount of time spent in wake or sleep. Furthermore, genetically obese animals, such as leptin-deficient mice and Zucker rats also show disruption in the amount of sleep and sleep timing. Taken together, these findings suggest that there is likely a bidirectional relationship between obesity, sleep, and circadian disruption.

Environmental Disruption and Metabolic Dysregulation

Thus far, we have discussed the proximate mechanistic links between circadian timing and metabolic function in terms of genetic mechanisms. However, clock gene mutations and tissue-specific knockouts do not directly address the ultimate causes of circadian disruption–induced metabolic dysregulation. In humans, genetic disruptions are likely not a significant driver of the links between obesity and circadian disruption. Instead, it is more likely that our disrupted circadian environments are the primary drivers. Epidemiologically, the "obesity epidemic" seems to be correlated with a gradual decrease in sleep time and quality. This correlational relationship does not directly imply causation, but it

is intriguing. In more focused studies, obvious circadian disruption is found in shift workers (eg, airline crews, truck drivers, medical doctors, law enforcement, and the military). Even in the general public, individuals are exposed to light late into the night (eg, TVs, tablets, smart phones), and have work schedules that may conflict with their endogenous chronotype (ie, circadian preference). This phenomenon has come to be known as "social jet lag," which describes the changes in sleep patterns from work to nonwork days (eg, holidays and weekends). This is significant, as individuals who report poor sleep show increased incidences of diabetes and risk factors for the development of cardiovascular disease, and social jet lag is associated with a higher BMI. In human laboratory studies, misaligned sleep can lead to plasma glucose levels approaching those of diabetics. However, the physiological and cellular mechanisms translating *environmental* circadian disruption to increased weight gain and metabolic syndrome remain unknown. This gap limits our understanding of the mechanisms by which environmental circadian disruption leads to metabolic dysregulation. To explore the mechanisms behind these effects, it is necessary to apply animal models in which we can manipulate the circadian environment (ie, the light–dark cycle) and determine how this affects metabolism.

Understanding the mechanisms whereby the SCN synchronizes peripheral clocks and is able to affect downstream pathways, such as glucose and lipid metabolism, has been a major focus for researchers in this area over the last decade. Since circadian rhythms are highly conserved among species, the findings from animal studies translate well to understanding the interconnections among the environment, circadian rhythms, sleep, and metabolic function in humans. For instance, altering light–dark cycles of rodents is an elegant and simple method to probe how circadian disruption affects myriad physiological systems. Inappropriate lighting (eg, light at night) has been used to explore some of these effects. Mouse studies show that exposure to dim light at night causes body weight increases, but no increase in food consumption. Similarly, using nonstandard lighting periods in mice (eg, 20 h days, 10 h light, 10 h dark cycles) causes weight gain, elevates plasma, leptin insulin, and triglycerides, and an increased insulin:glucose ratio. These are all indications of the metabolic syndrome, and represent a prediabetic or diabetic state. Both of these cases (light at night and 20 h days) do not cause an increase in overall food consumption, but instead lead to a reorganization of feeding behavior. This suggests that disruption of these feeding rhythms may be a crucial component for development of circadian-induced obesity.

CIRCADIAN DISRUPTION AND COGNITIVE FUNCTION

Circadian rhythms are a remarkable feat of biological complexity. Perhaps more remarkable is the fact that in most cases we are generally oblivious to their daily operation until we engage in a round of working night shifts, or try to function while severely jet lagged. After these acute circadian disruptions, changes in mood, affect, or cognitive function are commonly reported. The neurobehavioral dysregulation caused by circadian disruption has had some stark consequences, as several of the most notorious industrial accidents in the past few decades, including the Bhopal disaster in India, the Chernobyl nuclear accident in Ukraine, and the Exxon Valdez oil spill in Alaska occurred during the night. It is thought that several factors, including fatigue, interacted in each of these cases to cause or exacerbate the chain of events that lead to catastrophe. Therefore, the evidence is suggestive that disrupted circadian clocks and sleep cycles, coupled with high cognitive workload, could lead to significant performance deficits.

Circadian Effects on Learning and Cognition

While we may be extremely interested in the consequences of disrupted clocks in human performance, most human studies do not allow for mechanistic or causal experiments to be undertaken and basic research in animals can prove helpful. Many studies have demonstrated that circadian rhythms are important for normal learning and cognitive function. Even in simple invertebrates, such as the sea slug *Aplysia*, circadian effects on learning can be observed. In mammals, ablation of the SCN clock affects learning and memory. Hippocampal-mediated learning and memory has been particularly well studied, with clear circadian effects on spatial navigation tasks and object localization tasks, for instance. It is also known that the circadian clock influences hippocampal long-term potentiation (LTP), and aspects of the intracellular MAPK signaling pathway in hippocampal cells, which is essential for plastic changes that occur during learning, are modulated by circadian time.

Modeling Circadian Disruption by Unusual Light Conditions

Not only the evolutionary ubiquity of circadian clocks, but also their role in learning and memory (both simple and complex), underlie the significance of these phenomena and their importance in an organism's survival. Thus, modeling the effects of circadian disruption on learning, memory, and cognitive function is crucial. One such method involves using unique or "exotic" lighting conditions to drive circadian disruption. For instance, using a sequence of light pulses and a phase shift to cause arrhythmia in hamsters also impairs hippocampal learning and memory. Repeated jet lag models, where light cycles are rapidly shifted forward and back have also been helpful in this regard. As an example, chronic "jet lag" by repeatedly phase shifting the light–dark cycle results in learning and memory deficits that are accompanied by reductions in hippocampal neurogenesis. Exotic light–dark cycles can also provide important information, as chronically housing mice in shortened 20 h days leads to atrophy of neurons in the prefrontal cortex, and significant degradation of cognitive flexibility. Though the mechanisms are still unknown, accumulating evidence supports a role for circadian disruption as a causative contributor to neurocognitive deficits.

CIRCADIAN DISRUPTION AND PSYCHIATRIC DISORDERS

In many psychiatric disorders, a common comorbidity is disruption in the circadian sleep–wake cycle. By many reports, this is also one of the most significant quality of life issues. What remains unclear is whether disrupted circadian rhythms are a *symptom* or a *cause* of psychiatric disease. While it is unlikely that circadian rhythm disruption could cause de novo development of psychiatric disorders, much research suggests that disrupted clocks could shift probability of their occurrence in already vulnerable individuals.

Depression

Depression is a normal behavior in some instances, but it becomes pathological when it interferes with life, making it impossible for an individual to carry out daily activities. In addition to the classic symptoms of flattened affect and anhedonia, depressed individuals also report sleep and circadian abnormalities, including both insomnia (inability to sleep) and hypersomnia (excessive sleep and sleepiness). In addition to these behavioral outputs,

new evidence also shows that circadian clock gene polymorphisms are common in major depressive disorder, and that intensity of major depressive symptoms are correlated with circadian misalignment. Specifically, having a circadian pacemaker more delayed relative to sleep onset is associated with more severe depressive states. These types of data show us that disrupted clocks are associated with depressive disorders, but we still need to explore if this is a causal connection. Such studies are difficult in humans, but there are some populations that can help us unravel this mystery. For instance, shift workers often suffer from mood disturbances, including increased risk for depression. Again, animal studies can provide some additional information about these relationships. At the level of the molecular clock, mice with mutations in several clock genes (*Per*, *Cry*, *Bmal*) show increases in depressive-like behaviors. Conversely, manipulations that are known to cause increased depressive-like behaviors, such as different forms of chronic stress, can lead to abnormal rhythms of clock genes in brain areas associated with depressive-like behaviors, including the amygdala and hippocampus. At the circuit level, as mentioned earlier, chronic circadian phase shifting leads to decreased hippocampal neurogenesis, and reduced neurogenesis is associated with depressive-like behaviors in many animal models. In addition, atrophy of prefrontal cortical neurons that occurs in chronic housing in 20 h days is similar in extent and magnitude to decreases observed in chronic stress, which is also related to increased susceptibility to depressive-like phenotypes in animals. Looking at the entire landscape of findings, it seems clear that circadian disruption can contribute to depression, but unraveling symptomology and etiology is extremely difficult. Though it is clear that there is strong relationship between circadian disruption and depression, the effects are likely bidirectional.

Mania and Bipolar Disorder

A seemingly opposite behavioral state to depression is mania, and there are conditions such as bipolar disorder that involve rapid cycling between these two states. Connections between circadian rhythms, mania and bipolar disorder are an active area of research. It is well known that in humans manic episodes result in significant alterations of sleep patterns. In concert with the behavioral sleep abnormalities, several normally rhythmic physiological functions, such as body temperature and plasma hormone levels, are blunted during these episodes. Here again, use of the well-characterized molecular clock genes can help to assess causal connections. For example, mutations in the core clock gene *Clock* can lead to mania-like behaviors. Site-specific knockdown of *Clock* in the ventral tegmental area of mice can also induce manic-like behaviors, including increased locomotor behaviors, and altered responses in novel environments. In addition to effects of clock gene mutations on manic behavior, there are interesting examples in the experimental literature indicating that treatments known to reduce manic symptoms can affect clock mechanisms. Treating hamsters with lithium chloride (one of the most common treatments of bipolar disorder) causes a lengthening in the circadian period. Follow-up work in several species further indicates that lithium modifies many intracellular signaling cascades, including glycogen synthase kinase-3beta, which is an important link to cellular circadian mechanisms. Specifically, lithium indirectly inhibits GSK3β which then results in increased transcription of *Per2*. Thus, a pharmacological treatment that helps control symptoms of mania and bipolar disorder can have direct effects on circadian processes at the behavioral and molecular levels. Therefore, there is very strong evidence that disruption of the circadian clock is a component of some manic or bipolar

phenotypes. Furthermore, modification of the molecular clock can recapitulate many of these phenotypes, and treatments that reduce symptoms can directly interact with the molecular clockworks.

Schizophrenia

Over the past decade, links between normal cognitive function, depression, and mania and disrupted circadian timing are becoming clearer. However, schizophrenia has been a more difficult psychiatric condition to probe, both in terms of potential mechanisms and findings in clinical populations. Obviously, a contributing issue to this dearth of solid evidence is that the causes of schizophrenia are unknown, and the fact that the disorder is likely a consequence of both environmental and genetic factors. Even with this added complication, there is some evidence of links between circadian timing and schizophrenia. First, schizophrenic patients show clear fragmentation of rest–activity cycles. Second, sleep problems are also evident, with people suffering from schizophrenia showing both sleep onset and sleep maintenance insomnia. Finally, melatonin, a key hormonal regulator of sleep in many mammals including humans, shows a disrupted rhythm in schizophrenia sufferers. Taken together, the evidence points to disruption of multiple systems that are normally under tight circadian control. There is a genetic contribution to development of schizophrenia, and excellent data exist on the genetic makeup of several human populations. In one such study, polymorphisms in the vasoactive intestinal polypeptide 2 receptor (*VIP2r*) are associated with increased risk for development of schizophrenia. That these polymorphisms are in *VIP2r* is particularly interesting because VIP and its receptors (including *VIP2r*) are very important in the network functioning of the SCN clock. This represents an intriguing link between a human polymorphism associated with schizophrenia risk and a noncore "clock gene." Animal models of schizophrenia are also being actively explored for potential circadian and sleep abnormalities. For instance, the "blind drunk" (Bdr) mouse model of schizophrenia-like symptoms show fragmented circadian rhythms, as well as a phase-advanced period under entrained conditions. So while the links between circadian rhythms and schizophrenia are not as clear as in depression or manic behaviors, there is nonetheless compelling evidence that alteration of circadian timing systems may increase the risk of developing schizophrenia.

SUMMARY AND CONCLUSIONS

Disrupted circadian clocks are observed in many psychiatric conditions including depression, mania, and schizophrenia. While it may be easy to dismiss these changes as merely symptoms of the disorders, or even consequences of their treatments, it is also intriguing to think about the role that circadian rhythms may play in development of such disorders (Fig. 27.4). As mentioned at the outset of this section, it is highly unlikely that circadian disruption is the sole factor causing these disorders to develop, especially since they are usually polygenic, and are influenced by experiential and environmental exposures. However, it is likely that disruption of the clock somehow alters an individual's risk. This is an active area of research, enabled by the current focus on "personalized" treatment protocols that are sensitive to differences among individuals. Thus, while it is still unknown if disruption of circadian rhythms is a symptom or an etiology of psychiatric disorders, they clearly play an important interactive role, and understanding how they interact with other known mechanisms may provide additional insight in the causes of mental disorders.

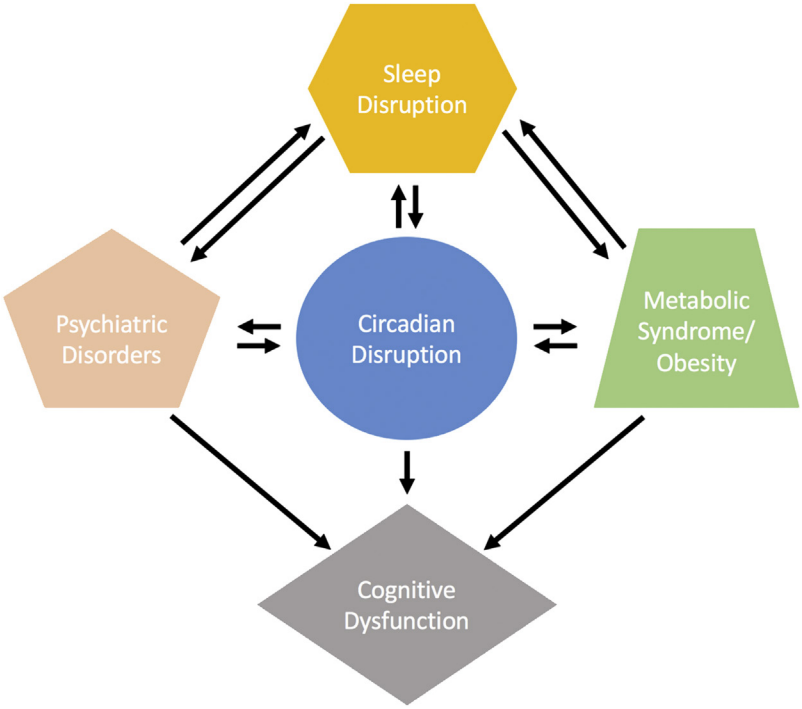

FIGURE 27.4 Summary of the interplay between circadian disruption and sleep disruption, and the subsequent effects on mental and physical health. Note some relationships are bidirectional, while other relationships seem more unidirectional. There is substantial evidence that circadian and sleep disruption can have wide-ranging effects on both mental and physical well-being.

Further Reading

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