





WHEN THE BODY'S CLOCK IS OFF, MENTAL  
HEALTH SUFFERS | BY SARAH C. BALDWIN

# OUT OF SYNC

Colleen McClung's love affair with neuroscience began in the late '90s, when she was a graduate student at the University of Virginia. A professor recommended she rotate in the lab of Jay Hirsh, a biologist who was studying the neuro-development of *Drosophila melanogaster*—specifically, the behavioral effects of cocaine on the flies' dopamine receptors. To do that, they first had to figure out how to get fruit flies to take the drug. Their solution? Freebasing. “We vaporized the cocaine,” says McClung, a PhD. “When the flies inhaled it, they went nuts—twirling around in circles, walking backwards. It was fascinating!”

In 1998, McClung and Hirsh published a paper in *Current Biology* establishing *Drosophila* as a viable model for studying behavioral responses to cocaine in humans. Around the same time, a fellow graduate student in the lab had made another fruitful fruit-fly finding, which subsequently appeared in *Science*: genes that control circadian rhythms are also implicated in sensitization, a process whereby repeated exposure to a drug increases the intensity of response.

Researchers at Pitt are bringing the mysteries of our circadian system to light. Their discoveries about its role in reward-seeking, decision-making, and mood disorders could help us keep our bodies in tick-tock, er, tip-top shape.

ILLUSTRATION | STACY INNERST

This discovery sparked McClung's interest in the role of circadian genes in behavioral and neuronal responses to drugs of abuse. (At the time, few labs were studying this connection.) During her postdoctoral fellowship at the University of Texas Southwestern Medical Center, she began to explore the genes that make drugs rewarding—this time, in mice. When she looked at mice with mutations in their circadian genes, she found that their response to drugs was altered, just as the flies' had been.

The circadian system—the myriad gears that synchronize body clocks—can affect us in profound ways. Scientists are still trying to grasp the extent of it, but they've learned that disruptions to our body rhythms can make us more vulnerable to addiction and to serious psychiatric illnesses like depression and bipolar disorder. Today, McClung is an associate professor of psychiatry and of clinical and translational science at Pitt, where she and colleagues are decoding the complex interplay between our brains and our inner metronomes. Their findings are turning the clock forward on new approaches to intervention and treatment.

The human circadian system is composed of the suprachiasmatic nuclei (SCN), a pair of small structures tucked in the hypothalamus that act as the brain's central timekeeper,

ing down the optic nerve to the SCN, starting the cycle over again.

In other words, the circadian system is the boss of us. It calls on both internal cues (the clocks in our brain and our cells) and environmental cues (zeitgebers, or “time-givers,” such as temperature and light) to tell our bodies what to do and when: Eat. Get some shut-eye. Wake up and hunt down a woolly mammoth.

## DYSREGULATION'S DANGERS

A healthy circadian rhythm means rising when it's light out and going to sleep when it's dark. When our circadian rhythms are out of sync with the outside world, the results can be uncomfortable (think jet lag, when your body believes it's 9 p.m. but the clock in your Paris hotel room says it's 3 a.m.) or, over the long term, disastrous, causing adverse health effects ranging from diabetes to depression.

Babies with disrupted rhythms are more likely to experience anxiety in their early teens, studies have shown. Women who sleep poorly during weeks 10 and 20 when pregnant have more complications. And elders are vulnerable, too: McClung recently published a paper revealing startling changes in the circadian system later in life. In a postmortem study of gene expression in the brains of 146 people, she and colleagues found that the rhythm in the clock

puberty brings with it more than just pimples and periods. It also shifts circadian rhythms backward, so that adolescents naturally stay up until the wee hours and sleep in late in the morning.

At least they wish they did. In reality, they face, in McClung's words, “the environmental risk factor that is high school.” Teenagers are simply not made to be sitting in a classroom at 8 in the morning. And this clash is exacerbated by yet another complex and all-important process, one that circadian rhythms modulate: reward function. It's the collision of these systems that makes teens acutely vulnerable to problems with mood, substance use, and even addiction.

## PLEASURE SEEKING

We experience reward via the brain's mesolimbic pathway. Dopamine, a neurotransmitter that mediates motivation for pleasure, journeys down this path from the midbrain's ventral tegmental area, to the nucleus accumbens in the forebrain's ventral striatum. It's part of our age-old survival system, enabling us to know—and remember—what we find pleasurable so that we pursue it again. This pertains to natural stimuli like food, sex, and social interactions, and also to drugs.

Like McClung, Brant Hasler, PhD assistant professor of psychiatry and psychology,

**When our circadian rhythms are out of sync with the outside world, the results can be uncomfortable (think jet lag, when your body believes it's 9 p.m. but the clock in your Paris hotel room says it's 3 a.m.) or, over the long term, disastrous, causing adverse health effects from diabetes to depression.**

and the genes that make up the biological clock, which are found in almost every cell of the body. The SCN contains thousands of neurons that fire rhythmically in response to light and darkness. The circadian genes in our cells produce clock proteins, whose levels rise and fall on an approximately 24-hour cycle, triggering or suppressing a host of key physiological processes: blood pressure, heart rate, digestion, hormone production, and appetite. The SCN's claim to fame, of course, is regulation of the sleep-wake cycle. Every morning, daylight resets our biological clock by travel-

genes of older people had slowed—588 genes showed a complete loss of rhythmicity. This, she says, might explain some of the alterations that occur in sleep, cognition, and mood in our later years. Interestingly, in brains of people older than 60, they also found a set of genes that gained rhythmicity, indicating the possibility of “some kind of compensatory clock that kicks in,” says McClung, who plans to investigate this in future studies.

But if there's one group that has become the poster children for chronic desynchronization, it's teenagers. We know now that

explores how the circadian and reward systems interact at the neurobehavioral level. Hasler, who's on the faculty of Pitt's Sleep and Chronobiology Center, wants to understand exactly how that interaction makes kids more likely to overindulge in drugs and alcohol. In McClung's lab, they've created a sort of rodent high school, where adolescent rats are placed on a treadmill in the morning Monday through Friday and allowed to live according to their natural circadian rhythms on the weekend, paralleling the lifestyle of human teens.





From substance abuse to speeding, our body's internal clock influences a lot more than snoozing.

“One of my working models is that adolescents are suffering from circadian misalignment, also known as social jet lag, where they’re forced to adopt early schedules during the week and then shift to the later schedule on the weekend,” Hasler explains. “They’re bouncing back and forth, traveling across several time zones Sunday night to be ready for Monday morning. This has a number of consequences, including the way we process reward.” The findings of the teen rat study, presented this May at the meeting of the Society for Research on Biological Rhythms, suggest that the reward process in the socially jet-lagged teen is, indeed, altered.

To shed more light on how circadian rhythm disturbance and impaired reward function conspire to make young people more susceptible to substance abuse, Hasler is running a novel study in which he’s deliberately putting teenagers’ rhythms out of whack. During the summer, healthy teens will make two visits to the sleep lab. Each time, they will have spent

a week staying up until midnight and rising at 9:30 in the morning—in other words, obeying their body clock. On the “aligned” visit, they’ll stay on the midnight to 9:30 schedule for a night; on the “misaligned” visit, they’ll follow a week of “natural” rhythms with an 8 p.m.–5:30 a.m. “sleep opportunity.” Each of these will be followed the next day by fMRI tests to measure how reward-related activity in their brains has changed.

Another study, which Hasler calls a “natural experiment,” looks at actual drug and alcohol use among college students, who, like high schoolers, regularly cycle through the havoc-rearing weekday-weekend transition. “We expect that, as their sleep and circadian timing change, their alcohol use behavior will, too,” he says. “On Thursday we measure levels of melatonin—a hormone of darkness, signaling biological night.” Melatonin, a well-validated marker of circadian timing, enables researchers to gauge what time it is according to each young adult’s body clock. “We put them in an

fMRI on Friday morning to measure brain response to a monetary reward task. Then they’re free to go do whatever they’re going to do on the weekend—presumably smoke pot and drink.” On Sunday night they come in for another melatonin assessment, followed by a Monday morning fMRI. “We’re asking, *Does circadian timing predict how their brains anticipate and respond to reward?*”

One of Hasler’s mentors on the misalignment study, clinical and developmental psychologist Erika Forbes, is also keenly interested in reward function in adolescents as it affects mood and addiction. Forbes, who directs Pitt’s Affective Neuroscience and Developmental Psychopathology Laboratory and is a PhD associate professor of psychiatry, psychology, and pediatrics, says that the teen brain does not fit on a smooth developmental continuum from childhood to adulthood. “In this period, things look dramatically different from the stage before and the stage after it. Teens are more distracted by pleasant stimuli when



they're engaged in a task, and they show more response in reward areas when there is a reward. They engage in more reward-seeking behaviors—driving fast, trying drugs, doing the foolish things they know better than to do. That's reflected in changes in the brain's reward circuitry."

As in McClung and Hasler's work, the parts of the brain that are key to much of Forbes's research are the ventral striatum, a stop along the reward pathway, and the medial prefrontal cortex, or mPFC, which mediates decision-making about reward—as in, *Should I drink that beer?* Hasler, in collaboration with Forbes and Department of Psychology Chair Daniel Shaw, has found that adolescent evening chronotypes—night owls—are more likely to have problems with alcohol. When anticipating a reward, they show less mPFC reactivity (in other words, their decision-making skills aren't great), while showing more ventral striatum activa-

Laboratory of Neurocognitive Development, is studying how the adult brain recruits several regions to enhance cognitive control of behavior, while the adolescent brain uses a different pattern of function.)

Because the neural reward system is still developing during the teen years, Forbes says, it's more vulnerable to becoming dysregulated. But that also opens the door to intervention. "As a developmentalist, I love to think there are these moments of opportunity to get in there to take advantage of the brain's plasticity and change things in a positive way."

Four years ago, Forbes published a study with neuroscientist Mary Phillips, an MD/MD (Cantab), who is an expert in neuroimaging technologies, holds the Pittsburgh Foundation-Emmerling Chair in Psychotic Disorders, and is professor of psychiatry and of clinical and translational science. That study was the first to reveal that circadian genes affect the neural circuitry of reward. The

associated with these processes—as well as the white matter connecting them.

Phillips and McClung are using a mouse that simulates human mania via a mutated circadian gene, given to her lab by renowned neurobiologist Joseph Takahashi, to understand the neurocircuitry of one phase of bipolar disease (the mice don't get depressed).

"They're hyperactive and impulsive like bipolar patients in manic phase, who often go on shopping or gambling sprees or abuse drugs. Anything that's rewarding, these mice find more rewarding," McClung says.

### GETTING ON PACE

Phillips, with professor of psychiatry and psychology Alison Hipwell, a PhD, is also scanning the brains of 3-month-old babies to get baseline data on early neurocircuitry and temperament. "It could be that between 3 and 9 months something crucial happens that determines a human being's tempera-

**In McClung's lab, they've created a sort of rodent high school, where adolescent rats are placed on a treadmill in the morning Monday through Friday and allowed to live according to their natural circadian rhythms on the weekend, paralleling the lifestyle of human teens.**

tion during the reward (which means they get more pleasure from the beer). This combination is associated with increased drinking problems. The group has also found that the weekday-weekend shifts in sleep cycles are associated with less reactivity in both regions, possibly contributing to substance abuse and depression: some teens need more of the drug or alcohol to feel good, and their ability to feel good at all is compromised.

The problem is not just with drugs and alcohol. "Adolescents are more sensitive to social context, too, especially with peers or friends," Forbes points out. "Kids drive in a riskier way when they have friends in the car. Risky sexual behavior or substance use is happening in a social context, too. It's not a kid alone in his room, it's a kid trying to get status or who's just excited by being around friends." (Of course, these behaviors are not all attributable to dysregulated rhythms. Pitt's Beatriz Luna, a PhD and the Staunton Professor of Psychiatry and Pediatrics who directs Pitt's

two continue to use fMRI to study adolescent-specific reward processing, peering deep into the brains of both healthy and mood-disordered teens, as well as those who don't yet have a mood disorder but who might develop one. (Research has shown that children of depressed or bipolar parents are more prone to develop the disorders themselves.) Their work indicates that lack of sleep, coupled with the dampened response to reward, could intensify some teens' quests for pleasure.

Phillips is particularly interested in comprehending the neural underpinnings of bipolar spectrum disorder. Patients with the disorder are known not only to have a disturbed sleep-wake cycle, but also to process emotion and reward abnormally. They all go together, she says. "People who have aberrant circadian function also tend to be hypersensitive to reward and therefore are highly thrill seeking." She uses a variety of neuroimaging techniques to "drill down" and observe in real time the brain regions and structures

ment—and that can be changed," she says. "The younger you are, the more malleable the brain is. The brain is developing until age 25, in terms of white matter maturation. So if you can go in and do something to right a wrong or change the path in a good way, that could stop a lot of suffering." While that's a ways off, Phillips says that we can use this knowledge "to guide strategies to help improve longer-term outcomes in otherwise disadvantaged young children."

This is not work for a lone wolf, notes Phillips.

Plumbing the seemingly infinite mysteries of the human brain to improve therapeutic and preventive measures requires multiple perspectives, she says: "People get very focused on their own area and they drill down and drill down, and they don't put their head above the parapet and see what's out there. We have to think about the overlaps, the similarities. ... Until you talk to everyone it's difficult to get the big picture." ■