SEEKING INSIGHTS INTO CIRCADIAN BEHAVIOR & ATMOSPHERIC POLLUTION

Assistant Professor Casey Diekman, Department of Mathematical Sciences, and Assistant Professor Alexei Khalizov, Department of Chemistry and Environmental Science, are working to expand the boundaries of our knowledge on two very different scientific frontiers. But a common denominator is the recognition and support each has received that highlights the significance of their efforts–support which includes Faculty Early Career Development (CAREER) grants from the National Science Foundation (NSF). CAREER grants are among the NSF's most prestigious awards, offered specifically for the benefit of younger faculty who, in building their careers, have demonstrated outstanding potential as both educators and researchers.

DELVING DEEPER INTO CIRCADIAN RHYTHMS

Diekman is studying the circadian rhythms that harmonize our behavior with the daily cycle of light and dark, and with seasonal change. These rhythms are among the most powerful physiological forces that humans and many other living organisms experience each day.

Increasingly, our culture is also challenging the circadian imperatives programmed by evolution. It's why traveling quickly across multiple time zones causes jet lag, and why shift work can affect physical well-being. There may even be a circadian influence on why serious cardiac events are more likely to happen at certain times of the day, and why chemotherapy may be more effective if administered at certain times.

Since coming to NJIT in 2013, Diekman has worked to advance what we know about circadian behavior with new tools for modeling circadian processes at cellular and behavioral levels. In addition to his recent five-year continuing CAREER grant, with some $115,000 awarded to date, Diekman's funding includes another NSF grant of more than $233,000. This substantial support for his research could also contribute to the overall success of the U.S. BRAIN Initiative — a program acronym for Brain Research through Advancing Innovative Neurotechnologies.

BEGINNING AT THE NEURONAL LEVEL

Diekman continues to look at neuronal activity in the suprachiasmatic nucleus, or SCN, a brain component consisting of some 20,000 neurons in the hypothalamus. The SCN receives information about the daily light-dark cycle from the external world through the retina, input that can affect circadian behavior. "The job of this part of the brain is to know what time of the day it is," Diekman says. But the SCN can perform this function without direct light-dark exposure, and it is this capability that can be linked to conditions such as jet lag.

Diekman explains that this may be part of our evolutionary heritage, possibly the evolution of distant mammalian ancestors that spent daylight hours inactive in dark spaces, "instinctively" emerging at night to avoid predators and forage safely for food. "You want a system that is robust enough so that the internal sense of time will override confusing 'noise' in the environment, such as heavy cloud cover, yet respond appropriately to changes in the environment. So we experience jet lag because the robustness of the clock that serves us well under some circumstances also has 'inertia' which can cause us to lag in adjusting to environmental changes."

Our circadian behavior over the course of the day is the cumulative product of the interplay of ionic currents within SCN neurons that generate electrical activity. This activity, occurring on a millisecond time scale, is influenced by daily oscillations in gene expression within the SCN. Gene expression is the process by which DNA is translated into proteins, and proteins are the engines of most physiological functions, including circadian behavior.

Experimental neuronal data for Diekman's mathematical modeling comes from Professor Hugh Piggins and Research Associate Mino Belle at the University of Manchester in England. A key objective for Diekman and Matthew Moye, a graduate student in mathematical sciences at NJIT, is to integrate this data into a comprehensive physiological model that simulates neuronal activity on the order of microseconds and clarifies the role of various ionic currents in daily circadian patterns.

Assistant Professor Casey Diekman is investigating how humans and many other living organisms are naturally attuned to the time of day.

PHOTO: BRITTANI BRUNDAGE

ENLISTING *DROSOPHILA*

While Diekman still works with data generated by the circadian clock in mammals, he has enlisted a new laboratory ally — Drosophila, more commonly known as the fruit fly. In collaboration with Professor Ravi Allada and Research Associate Matthieu Flourakis at Northwestern University, he is studying the electrophysiology of circadian pacemaker neurons in Drosophila.

Diekman is continuing to develop models that mathematically detail the functioning of individual cells in the mammalian SCN. But constructing a more comprehensive picture of circadian phenomena requires insight into how the 20,000 cells of the SCN interact at the network level to influence behavior.

The circadian clock in fruit flies shares many characteristics with the mammalian system, Diekman explains. Yet there are just 150 neurons in a fruit fly's circadian neuronal network. This relative simplicity promises to facilitate investigation of phenomena such as how neurotransmitter signaling molecules enable individual cells to synchronize with each other in response to changes over the light-dark cycle. This will also entail modeling the distinctive characteristics of seven different groups of cells, or clusters, in the fly's circadian network and the functional connections among them.

"The goal is to build models of the complete fruit-fly clock, including detailed molecular and physiological models, and to test our models in the laboratory by measuring how long it takes the flies to respond to variations in the light-dark cycle," Diekman says. "We believe that experiments replicating travel across time zones, for example, will help to answer significant questions about entrainment, how our physiology and behavior synchronize with environmental cues or at times are more in synch with what our internal clock tells us."

In their 2015 paper in the journal *Cell*, "A conserved bicycle model for circadian clock control of membrane excitability," Diekman and researchers in Allada's lab reported that increased sodium leak conductance depolarizes Drosophila circadian pacemaker neurons in the morning, leading to high electrical activity. In the evening, increased potassium conductance hyperpolarizes these neurons and silences electrical activity. Remarkably, antiphase cycles in sodium and potassium conductances also drive membrane potential rhythms in mouse circadian clock neurons. Thus, this "bicycle" mechanism of controlling membrane excitability is an evolutionarily ancient strategy for governing daily sleep and wake behavior.

BROADER CIRCADIAN PERSPECTIVES

Diekman and Professor Amitabha Bose, an NJIT mathematical sciences colleague, are also developing a new mathematical tool for predicting the phase of our circadian entrainment called an "entrainment map." Their approach maps physiological responses to factors such as light intensity and seasonal changes in light duration to a mathematical function that determines the phase of entrainment.

Our phase of entrainment has significant implications reflective of both nature and culture. Most of us contend with jet lag only occasionally. A greater number of individuals regularly experience the effects of shift work. But delayed circadian adjustment to "springing ahead" only one hour for daylight saving time may have more general negative consequences indicated by the statistical spikes in traffic accidents and workplace injuries that occur at this time of year.

Diekman says he is also intrigued by research indicating that circadian rhythms are integral to the physiology of cells throughout our body, not just in the SCN. This could explain why certain cancers seem to be more susceptible to chemotherapy at particular times of the day, and why cardiac arrest appears to occur more frequently late in the morning and early in the evening.

Circadian rhythms are found not just throughout the animal kingdom, but in plants and certain types of bacteria as well. Collaborating with Professor Horacio Rotstein and graduate student Emel Khan from mathematical sciences, Diekman is also developing mathematical models of the circadian clock in

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cyanobacteria. These models will be used to generate hypotheses about entrainment mechanisms that will be tested in the laboratory of Professor Yong-Ick Kim, NJIT Department of Chemistry and Environmental Science.

RESEARCHING THE ATMOSPHERIC MYSTERY OF MERCURY

Mercury, as Alexei Khalizov emphasizes, presents a very serious environmental problem. We know that substantial amounts of this highly toxic element are released into the atmosphere through the burning of coal and petroleum for fuel and by the incineration of our civilization's garbage. We also know that mercury entering the atmosphere can eventually find its way into the soil and, especially, the world's oceans, where it poses a threat to health by accumulating in many species of fish that we eat.

But as Khalizov also explains, the chemical transformation of mercury released by combustion that takes place in the atmosphere as a precursor to dangerous contamination of soil and water is not clearly understood. It's a scientific mystery that Khalizov is working to solve with the support of his five-year NSF CAREER grant, which totals some $670,000.

MERCURY HAPPENED

Khalizov's path to NJIT was via the Russian Federation, where he earned his undergraduate and graduate degrees in chemistry. His first postdoctoral position brought him to Canada's McGill University, to an opportunity that involved researching atmospheric chemistry. This included investigating the chemistry of rapid mercury-depletion events in the Arctic atmosphere that led to significant inputs of oxidized mercury into the soil and water over a very short time span. This is a particular health concern for the region's indigenous people because of their dependence on fish as a staple food. "So in a sense, mercury 'just happened' with respect to becoming an important research interest for me," Khalizov says.

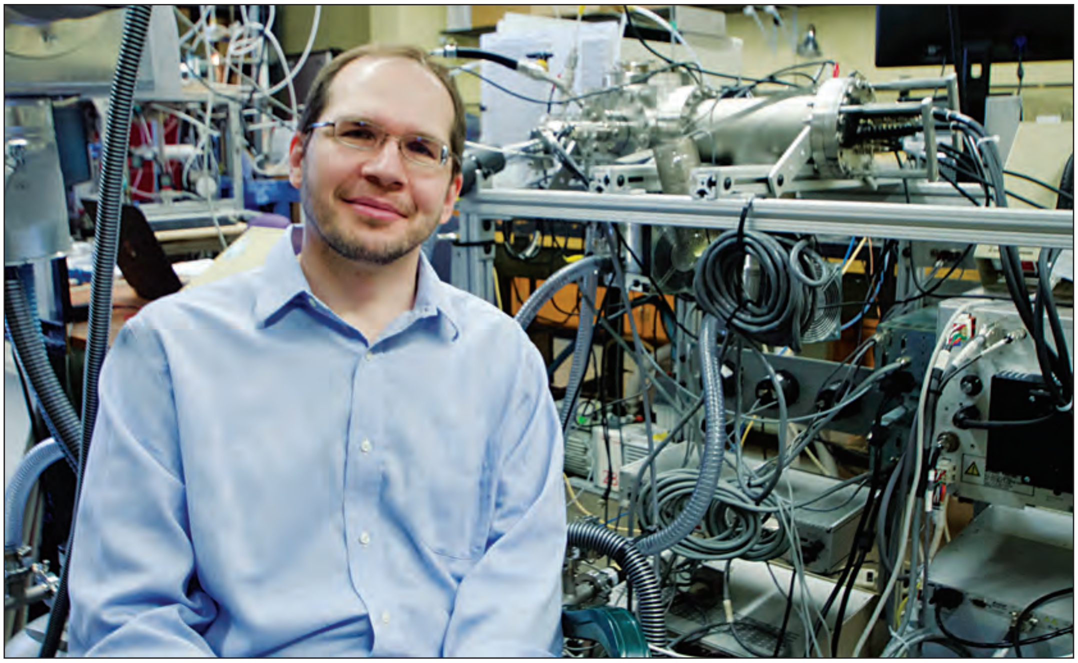

PHOTO: BRITTANI BRUNDAGE

Assistant Professor Alexei Khalizov with instrumentation he is developing to study atmospheric mercury contamination.

It's an interest that Khalizov pursued periodically during further research at Texas A&M University. Now at NJIT, which he joined as a faculty member in 2013, mercury again has become a major focus of his research. He also has been studying another pollutant, atmospheric soot, with substantial NSF funding. This aspect of his work was recently featured in an article in the journal *Geophysical Research Letters*, "An unexpected restructuring of combustion soot aggregates by subnanometer coatings of polycyclic aromatic hydrocarbons."

It is noteworthy that both soot and mercury pollution problems originate from humanity's heavy reliance on fossil fuel combustion for energy generation and transportation. "We can measure how much gaseous elemental mercury is released into the atmosphere as a result of combustion," Khalizov says. "However, we really don't understand the chemistry by which this form of mercury is oxidized and then becomes bound to particles in the atmosphere. These are the stages before the mercury becomes dangerous in the food chain, before rain and other forms of precipitation cause the oxidized mercury to enter the ocean and other bodies of water. We know that this process appears to occur in the atmosphere over one or two years, but chemical details are missing."

THREE MAJOR COMPONENTS

Going forward, Khalizov further explains that this research will have three major components. One will be investigating the relevant chemical reactions in the laboratory, particularly how gaseous elemental mercury is oxidized by reacting with bromine atoms to form a short-lived radical and how that radical is converted into stable molecules. This will require characterizing reactions that take place on a time scale of milliseconds. A second major objective will be to clarify how the resulting gaseous products attach to particles in the atmosphere.

Gaining this knowledge will require innovative measurement techniques and appropriate instrumentation, including instrumentation to measure oxidized atmospheric mercury in the field with accuracy comparable to that which can be achieved under controlled laboratory conditions. This is the third major component of the research effort that Khalizov is mapping, and he anticipates developing new instruments such as a chemical ionization mass spectrometer for detection of gaseous oxidized mercury and a desorption electrospray ionization mass spectrometer capable of chemical analysis of mercury in aerosol nanoparticles.

Khalizov, who says that he will also be collaborating internationally with colleagues in Canada and China, looks forward to very productive experimental investigation at NJIT. "Again, we know how much mercury is introduced into the atmosphere and what the sources are. But we don't understand the oxidation process very well.

"I hope to contribute to finding out the details of what happens in a complex chain of chemical events. We have opened the door to a new, very large field for research. It's research that can provide the concrete data we need to develop better models of how atmospheric mercury migrates to other parts of the environment, and how this migration might be affected by different strategies for mitigation." ■

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