croscope tip and the surface. Measurements made with submolecular-resolution noncontact atomic force microscopy revealed the structure of the cyclocarbon compounds (see the figure). By analyzing the bond order of cyclo[18]carbon, the authors unambiguously identified a polyynic as opposed to a cumulenic geometry, thus ending a debate that has persisted for decades.

Over the past decade, scientists have extended the synthesis of new carbon nanostructures from traditional in-solution and gas-phase organic chemistry to well-defined crystalline surfaces in ultrahigh-vacuum environments. On-surface synthesis makes use of a solid surface as a two-dimensional (2D) template and a catalyst to initiate chemical reactions between molecular precursors by exposure to heat, light, or electron irradiation. The reaction mechanisms in on-surface synthesis are often different from those in traditional organic synthesis. Bottom-up on-surface fabrication by covalent fusion of polycyclic aromatic hydrocarbons (the structural and functional building blocks) is a flexible and highly precise methodology for the tailored design of new all-carbon and carbon-rich nanomaterials with unusual electronic, thermal, and mechanical properties. For instance, control of the width and edge structure of graphene nanoribbons or the incorporation of heteroatoms allows for band gap engineering to design new semiconductor materials.

Thus far, scientists have used mostly metal surfaces as substrates to construct well-defined, low-dimensional carbon materials with atomically precise edge geometry [e.g., fullerenes (*6*), carbon nanotubes (*7*), and graphene nanoribbons (*8*)]. Instead of metal, Kaiser *et al.* used insulating films to stabilize the reactive precursors so that the authors could follow the reaction step-by-step through atomic manipulation (see the figure).

The success of on-surface synthesis is inherently connected to recent developments in high-resolution scanning probe microscopy. The single-molecule sensitivity down to individual atoms and bonds and the possibility of atom manipulation to induce chemical reactions distinguish scanning probe microscopy at low temperatures in ultrahigh vacuum from traditional methods for molecular structure elucidation. In particular, as shown by Kaiser *et al.*, noncontact atomic force microscopy with a carbon monoxide–functionalized tip enabled atomic-resolution imaging of single molecules (*9*) as well as measurement of their adsorption geometry and bond-order relations (*10*). Hence, this methodology is indispensable for identifying intermediates and products in on-surface reactions at the atomic scale and for providing details about structure, conformation, charge state, electronic structure, and aromaticity.

The authors demonstrated further that because of the high reactivity of cyclocarbons, interconnected cyclocarbon oxides could be created by coalescence of the building blocks with this use of atomic manipulation techniques. The intermediate and final structures displayed molecular loops, which represent substructures of 2D networks. Therefore, the new study might pave the way to the creation of elusive 2D carbon materials that are thermodynamically less stable than graphene. In such a monomer-to-polymer approach, the controlled cyclotrimerization of cyclo[18]carbon might be used one day to produce graphdiyne (*11*).

Graphdiyne is a 2D network of sp-sp^2–hybridized carbon atoms formed by inserting diacetylenic linkages between two benzene rings in a graphene structure (*12*). The material has a porous structure that allows the adsorption and diffusion of atoms, which is highly desirable for the design of next-generation batteries, hydrogen storage systems, and catalysts. Because of the presence of a natural band gap and high degree of π conjugation, graphdiyne is also a promising material for nanoelectronics. However, strategies for bottom-up synthesis of single-layer crystalline graphdiyne films and the characterization of their basic physicochemical properties remain elusive; scientists face numerous challenges resulting from the rich structural diversity of the graphdiyne family and the uncontrollability in alkyne coupling reactions (*13*). The work by Kaiser *et al.* illustrates the potential of combining organic synthesis with atomic-resolution scanning probe microscopy to answer long-standing questions in chemistry and to explore a new world of synthetic carbon allotropes with unprecedented properties. ■

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NEUROSCIENCE

Countering opioid side effects

A genetic screen in worms reveals a receptor target to battle opioid addiction

By **Nicole Mercer Lindsay**[1,2] *and* **Grégory Scherrer**[1,2,3]

The toll from opioid overdose in the United States now exceeds 45,000 deaths per year. Shockingly, more Americans die from opioid overdose than from motor vehicle collisions (*1*), and opioid overdose has become the number one cause of accidental death. Worldwide, two-thirds of drug-related deaths were a result of opioids, as reported by the United Nations 2019 drug report. As well as searching for opioid replacements, scientists are developing therapeutics to block the detrimental side effects of opioids, particularly addiction and fatal opioid-induced respiratory depression (OIRD) (*2*). Addiction and OIRD are a direct result of opioid activation of receptors that regulate neural circuits that control reward and breathing—circuits distinct from those that regulate pain (*3*). On page 1267 of this issue, Wang *et al.* (*4*) identify the orphan G protein–coupled receptor (GPCR) GPR139 as a regulator of opioid receptors and provide evidence that this receptor could be a useful therapeutic target to reduce opioid side effects.

Wang *et al.* turned to a model organism with a simpler nervous system than that in mammals, the nematode worm *Caenorhabditis elegans* (*5*). They developed an ingenious forward genetic screen to identify mutations that affect opioid receptor function. Fentanyl, morphine, and other abused opioids primarily act on the μ-type opioid receptor (MOR). MOR is a GPCR present on the cell surface. Deletion of *Oprm1*, the gene that encodes MOR, in mice demonstrated that

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MOR is responsible for the analgesic effects of opioids as well as the harmful addiction and OIRD (*6*, *7*). In rodents and humans, opioids have motor effects, altering locomotion and muscle tension (*8*, *9*). *C. elegans* does not express MOR and is unresponsive to opioids. However, the authors found that following introduction of a transgene to express mammalian MOR (tgMOR), fentanyl and morphine decreased locomotion in tgMOR *C. elegans* mutants. Wang *et al.* then induced random mutations in the tgMOR *C. elegans* population to identify worms resistant to fentanyl. They found ~900 mutations and chose to examine one of the affected genes, *frpr-13,* which encodes a GPCR.

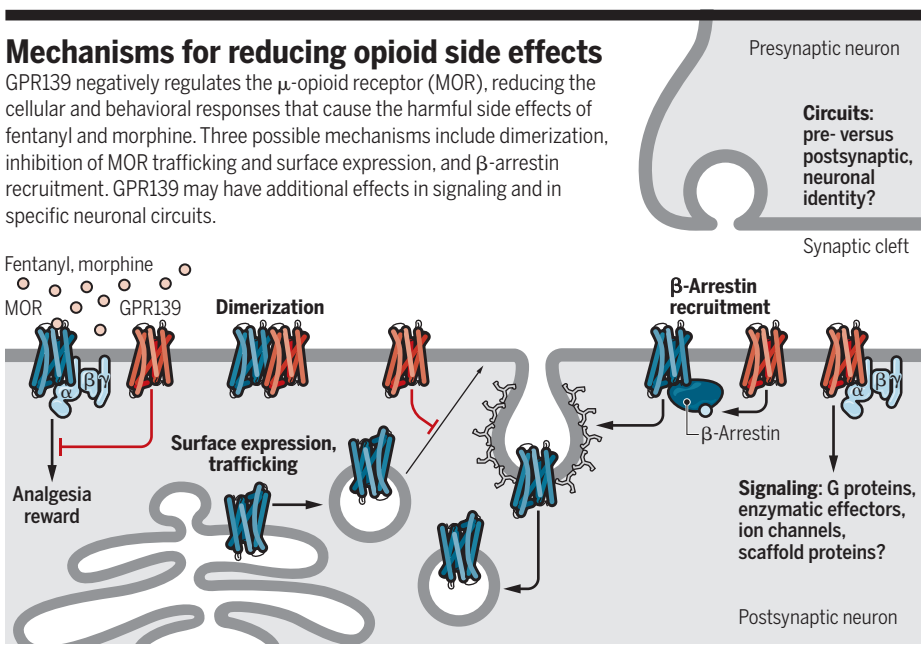
The mammalian ortholog of FRPR-13 is GPR139. Wang *et al.* validated the functional interaction of MOR and GPR139 by showing that MOR-induced membrane hyperpolarization, a known effect of opioids, was inhibited by GPR139 expression in cultured human kidney cells that do not usually express the GPCR. They next explored three possible molecular mechanisms by which GPR139 negatively regulates MOR function (see the figure). First, they explored whether GPR139 and MOR dimerization could explain this finding. They showed that GPR139 and MOR can be coimmunoprecipitated. However, both receptors were artificially overexpressed in a non-native cellular context; whether these interactions occur in vivo between endogenous receptors expressed at physiological concentrations and in the neurons that mediate opioid side effects remains to be determined. Second, when GPR139 is expressed at high amounts, they found that MOR is present at the cell surface in lower densities, suggesting that GPR139 may modulate *Oprm1* expression and/or MOR trafficking inside the cell, either its transport to the cell surface or its internalization. At stoichiometric amounts, GPR139 has no effect on surface localization of MOR, suggesting that this mechanism may only take place when GPR139 expression is upregulated, potentially in the setting of chronic drug exposure and disease. Third, the authors provided evidence that GPR139 negatively regulates MOR by facilitating β-arrestin 2 recruitment. β-Arrestins are cytosolic proteins that interact with GPCRs and promote receptor desensitization, internalization, trafficking, and signaling (*10*). β-Arrestin 2 is thought to contribute differentially to the multiple side effects of opioids (e.g., promoting OIRD, reducing reward without affecting withdrawal) (*11*, *12*) and may do so in an opioid ligand–dependent manner (*13*). Consequently, the exact opioid drugs and detrimental effects for which GPR139 modulation might prove useful will need to be established.

To investigate GPR139 function in neural circuits, the authors examined the medial habenula (MHb) and locus coeruleus (LC) of mice, two regions of the brain with neurons that express MOR, for electrophysiological evidence of MOR and GPR139 functional interactions. They found *Oprm1* and *Gpr139* mRNA colocalized in neurons in the MHb and LC. Using cultured brain slices, they found that the loss of *Gpr139* reduced the basal firing rate of MHb neurons and increased the opioid sensitivity of LC neurons. The authors conclude that GPR139 modulates MOR control of neuronal excitability by a cell-autonomous mechanism. However it seems that these effects could also result from an action of GPR139 in non–MOR-expressing neurons within the slice. Future studies should determine which ion channels are involved in the reduced neuronal firing; they should also establish whether GPR139 modulates MOR-induced inhibition of neurotransmitter release and probe GPR139 function in other brain nuclei of critical importance for opioid addiction, including the nucleus accumbens and the ventral tegmental area (*14*), and implicated in OIRD, such as the pre-Bötzinger complex (*15*).

Wang *et al.* investigated the importance of GPR139 in behavior. Mice in which *Gpr139* was deleted showed an increased sensitivity to morphine-induced reward and analgesia. They also examined the potential of GPR139 as a drug target. When mice were given morphine and then the GPR139 agonist JNJ-63533054, Wang *et al.* observed a reversal of morphine-induced analgesia. When the authors examined the behavioral effects of JNJ-63533054 using a reward test (self-administering morphine), morphine was less rewarding. Although JNJ-63533054 reduced analgesia and has unknown effects on OIRD, this result is exciting because it suggests that GPR139 targeting could potentially be used in the treatment of opioid addiction. More work is needed to clarify the translational potential of GPR139, starting with determining the coexpression of GPR139 and MOR in regions mediating addiction and OIRD in the human brain. It remains possible that GPR139 regulates other GPCRs besides MOR and that GPR139 has important functions in other brain regions and beyond, both of which could result in detrimental side effects following administration of an agonist. Although these questions are unanswered, Wang *et al.* have pioneered the use of forward genetic approaches with *C. elegans* in the opioid field, a technique that could be rapidly used to identify testable drug targets to combat the ongoing opioid epidemic. ■

Mechanisms for reducing opioid side effects

GPR139 negatively regulates the μ-opioid receptor (MOR), reducing the cellular and behavioral responses that cause the harmful side effects of fentanyl and morphine. Three possible mechanisms include dimerization, inhibition of MOR trafficking and surface expression, and β-arrestin recruitment. GPR139 may have additional effects in signaling and in specific neuronal circuits.



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