have their own contribution to the splice-switching efficacy, ribonuclease H activity, enzymatic stability, toxicity, protein corona formation, biodistribution, and delivery efficency (*13*). Enantiopure oligonucleotide phosphorothioates should also have improved efficacy.

Efficient chiral auxiliaries for the sulfurization step of the phosphoramidite coupling cycle have been developed (*9*), but their greenness and practicability for large-scale manufacturing are outstanding issues. For the P(V) platform, the purified isomers of limonene, a terpene extracted from citrus peels and used to produce chiral auxiliaries, can be prepared on a large scale. This feature, along with operationally simple and efficient coupling chemistry with a high degree of stereocontrol, makes the P(V) platform attractive for the production of enantiopure phosphorothioate oligonucleotides.

The next step for the P(V) platform will likely be to evaluate the scaling up of this process as well as the applicability of the platform for the liquid-phase technologies and compatibility with the alternative protecting group schemes. Although 4,4′-dimethoxytrityl (DMTr) works well as an acid labile 5′-protection for solid-phase assembly, severe problems are frequently encountered in solution because of the reversibility of the detritylation reaction and partial simultaneous acid-catalyzed deadenylation ("depurination") that occurs during deprotection. The simple coupling chemistry in the described P(V) platform combined with an atom-economic alternative protecting group scheme would be an attractive improvement for liquid-phase technologies and the sustainable production of oligonucleotides. ■

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NEUROSCIENCE

# Increased serotonin prevents compulsion in addiction

## Extracellular serotonin decreases cocaine taking in mice


*By* **Katsuhiko Miyazaki** *and* **Kayoko W. Miyazaki**


Drug addiction is a disease characterized by compulsive drug seeking and consumption despite negative consequences (*1*). About 20% of cocaine users lose self-control and are eventually diagnosed as addicted. Cocaine blocks dopamine, noradrenalin, and serotonin uptake through inhibition of the transporters of these neurotransmitters. The rewarding effect of cocaine is mainly induced by blocking the dopamine transporter (DAT), which increases extracellular dopamine concentrations. Intriguingly, mice that lack DAT still experience rewarding effects from cocaine, and this is abolished when both DAT and serotonin transporter (SERT) are deleted in mice (*2*, *3*). On page 1252 of this issue, Li *et al.* (*4*) show that increased extracellular serotonin antagonizes transition to compulsive cocaine intake by inducing a presynaptic depression in which serotonin inhibits excitatory synaptic transmission to the dorsal striatum (DS) through the serotonin receptor, 5-hydroxytryptamine receptor 1B (5-$HTR_{1B}$), which is expressed on the presynapse of cortical inputs.

For experimental animal studies in addiction, self-administration (SA) followed by punishment is commonly used (*5*). When cocaine SA subsequently becomes accompanied with punishment (such as foot shock), animals show two types of behavioral response. One is reduced cocaine SA (renouncers, ~80% of mice), and the other is continued cocaine SA (perseverers, ~20% of mice) (*6*). Direct causality between activation of dopamine neurons in the ventral tegmental area (VTA) and addiction was revealed by using optogenetic dopamine neuron self-stimulation (oDASS), which enabled inducible release of dopamine from the VTA (*7*). The use of oDASS results in a bimodal distribution of compulsive (perseverers, >50%) and noncompulsive (renouncers, <50%) individuals. Why is there a larger proportion of perseverers in the oDASS group than in the cocaine SA group?


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Li *et al.* found that cocaine SA in mice that express a SERT that blocks cocaine binding (SertKI mice) (*8*) does not increase extracellular serotonin concentrations, but unexpectedly, this causes increased transition to compulsion compared with that in wild-type (WT) mice. The population of perseverers of SertKI mice in cocaine SA is comparable with that of WT mice in the oDASS group. The authors also found that although oDASS-mediated addiction results in compulsion in more than half of individuals, systemic administration of citalopram, which is a serotonin-selective reuptake inhibitor, during acquisition of addiction through oDASS reduces compulsion transition to the same amount in cocaine SA of WT mice (~20% perseverers). These results indicate that increased extracellular serotonin prevents the transition to compulsion.

What neural and cellular mechanisms contribute to these serotonin effects? In oDASS-mediated addiction, perseverers were associated with increased neural activity in the orbitofrontal cortex (OFC), and chemogenetic inhibition of the OFC reduced transition to compulsion (*7*). Compulsive lever pressing in oDASS-mediated addiction was associated with increased neural activity in the projection terminals from the OFC to the DS, which is related to habitual drug use, and glutamatergic transmission from the OFC to the DS was permanently potentiated in perseverer mice (*9*). Consistently, Li *et al.* revealed that in both cocaine SA and oDASS groups, perseverers show synaptic potentiation of afferents from the OFC to the DS, which did not occur in renouncers. Bath application of serotonin to OFC-DS terminals in brain slices induced a presynaptic depression of excitatory transmission, which was prevented by the 5-$HTR_{1B}$ antagonist NAS181. Moreover, in cocaine SA-mediated addiction, ablation of 5-$HTR_{1B}$ (*10*) increases the proportion of perseverers to >50% because of the lack of the presynaptic depression.

Li *et al.* elegantly show that in WT mice, cocaine binds to SERT to block serotonin reuptake and increases extracellular serotonin concentrations, causing the presynaptic depression (see the figure). When 5-$HTR_{1B}$ at OFC neuron terminals projecting to the DS is activated, synaptic transmission in the


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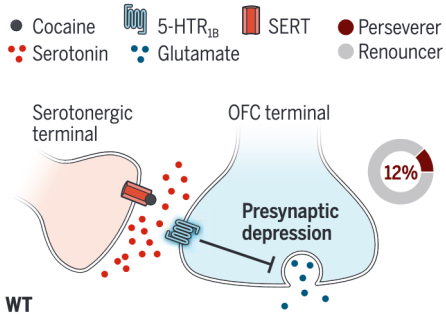
OFC-DS pathway is reduced by the presynaptic depression. Because enhancement of glutamatergic OFC-DS synaptic transmission is causally related to transition to compulsion in addiction, 5-$HTR_{1B}$-induced presynaptic depression prevents WT mice from transition to compulsion. In SertKI mice, because cocaine cannot bind to SERT, extracellular serotonin concentrations do not change. The OFC-DS synaptic transmission in SertKI mice is more efficient than in WT mice, and this induces increased transition to compulsive cocaine taking. When 5-$HTR_{1B}$ is ablated in mice, although cocaine binds to SERT and extracellular serotonin concentrations increase, the presynaptic depression at OFC-DS synapses does not occur because of lack of 5-$HTR_{1B}$. Increased OFC-DS synaptic transmission by cocaine SA thus induces increased perseverers in these mice.

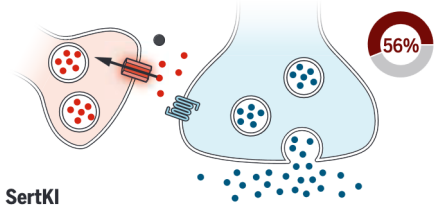
The study of Li *et al.* poses further questions regarding mechanisms of compulsion in drug addiction. After initial acquisition before punishment, are mice already shaped as renouncers or perseverers with different strengths of OFC-DS transmission, or do these two behaviors differentiate during relearning with punishment? Does transition to compulsion occur deterministically or stochastically? Does serotonin prevent transition to compulsion in taking other addictive drugs, such as opioids? Addictive drugs commonly increase dopamine in the mesolimbic pathway, and this would play a crucial role in the induction of compulsion in addiction (*11*). Further studies will clarify whether activation of 5-$HTR_{1B}$ during addictive drug intake can prevent transition to compulsion. Compulsive cocaine-taking individuals may show increased neural activity in the OFC, as in the case of oDASS-mediated addiction. In addition to affecting OFC-DS transmission, serotonin may modulate OFC neural activity to shift perseverers to renouncers in rats (*12*). Forebrain serotonin depletion induced compulsive cocaine seeking in rats, and systemic injection of a 5-$HTR_{2C}$ agonist reduced compulsive cocaine seeking (*12*). Serotonergic modulation in the OFC was also reported in a reward waiting task (*13*) and in reversal learning (*14*), indicating that a common mechanism by which serotonin regulates OFC neural activity may exist. Further studies should clarify the neural mechanism underlying serotonin modulation of the transition to compulsion in drug addiction, what agents specific to serotonin receptors can be used, and when these agents can be administered to potentially treat drug addiction. ■

## Serotonin inhibits addiction

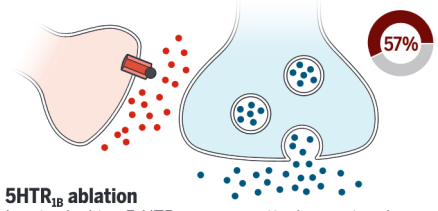
Extracellular serotonin antagonizes the transition to compulsive cocaine intake in mice by modulating glutamatergic transmission in the dorsal striatum.




**WT**
In wild-type (WT) mice, cocaine inhibits the serotonin transporter (SERT), which increases extracellular serotonin. This weakens orbitofrontal cortex (OFC)–dorsal striatum excitatory transmission (presynaptic depression) owing to activation of the serotonin receptor [5-hydroxytryptamine receptor 1B (5-$HTR_{1B}$)] on OFC neurons. This reduces compulsive cocaine intake by mice.




**SertKI**
In mice with a SERT mutation that prevents cocaine binding (SertKI mice), 5-$HTR_{1B}$ is not activated owing to lack of extracellular serotonin. Thus, enhanced OFC–dorsal striatum excitatory transmission remains, resulting in a high proportion of perseverers (mice showing compulsive cocaine intake).




**5$HTR_{1B}$ ablation**
In mice lacking 5-$HTR_{1B}$, presynaptic depression does not occur because of lack of presynaptic 5-$HTR_{1B}$, and so the population of perseverers increases.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MOLECULAR BIOLOGY

# Getting droplets into shape

## Protein clusters at interfaces control sizes and properties of biomolecular condensates

*By* **Wilton T. Snead**[1] *and* **Amy S. Gladfelter**[1,2]

Condensed droplets of protein and nucleic acid regulate a variety of cellular functions, from signaling to RNA processing (*1*). Many of these biomolecular condensates display liquid-like properties and are expected to evolve in size over time (called coarsening) to form a single, large droplet at equilibrium with its surroundings (*2*). However, condensates in cells frequently remain as a dispersed emulsion of droplets that do not increase beyond a certain size and maintain distinct molecular identities despite sharing a common environment. Various mechanisms that restrict coarsening have been proposed, including physical barriers such as chromatin or actin networks that keep droplets apart and prevent fusion (*3*), active processes that suppress material transfer between droplets (called Ostwald ripening) (*4*), and "kinetic arrest" due to protein binding site saturation (*5*). On page 1218 of this issue, Folkmann *et al.* (*6*) find another regulator of droplet coarsening: nanometer-scale protein clusters adhered to droplet interfaces.

Although interfacial stabilizers are conceptually new in the study of biomolecular condensates, these materials are well established in other fields such as chemical engineering and food science (*7*). Specifically, liquid-liquid emulsions can be stabilized by materials that reduce surface tension, a key parameter that determines how quickly droplets coarsen. A common example is a surfactant or detergent that stabilizes oil-in-water emulsions. Interfacial stabilizers can also include solid particles, collectively called "Pickering agents." These materials were originally described as stabilizers of inorganic emulsions (*8*) and are now used in diverse applications such as drug delivery, in which solid particulates composed of biocompatible polymers stabilize drug-carrying emulsions and assist with the controlled timing of drug release (*7*).

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