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Accessories to Addiction: G Protein Regulators Play a Key Role in Cocaine Seeking and Neuroplasticity

The prefrontal cortex mediates many aspects of addiction. In this issue of *Neuron*, Bowers et al. demonstrate that an activator of G protein signaling (AGS3) is persistently upregulated in the prefrontal cortex after cessation of chronic cocaine treatment. Furthermore, they find that AGS3 is responsible for altered behavior, such as enhanced drug seeking, and altered neurotransmission in cocaine-treated rats, representing a novel therapeutic target.

Drugs of abuse act on specific neurochemically coded brain circuits that normally serve to guide adaptive behavior and to maximize fitness and survival. In addiction, compulsive drug taking derails the brain's exquisitely tuned molecular signaling systems, altering the "set point" of motivational and reinforcement networks. This knowledge, provided largely by use of animal models of addiction, has accrued in conceptual stages with regard to precisely how external chemicals interact with chemical substrates in the brain. For example, early research in the past half-century focused on the behavioral and pharmacological effects of drugs, followed by descriptions of specific receptors for drugs such as opiates, cannabinoids, cocaine, and nicotine. In the past decade, molecular biological tools have enabled scientists to make the "transmembrane" leap, gaining knowledge about how addictive drugs affect intracellular signaling mechanisms and gene regulation (Nestler, 2001). The integration of these studies with a systems-based approach to the study of behavior and neural circuitry has led to major conceptual advances in how we think about addiction, particularly with regard to its long-term consequences for cognitive processing and emotional regulation (Everitt et al., 2001).

Some of these advances have resulted from applying discoveries in other fields to the problem of addiction, an approach taken by Bowers and colleagues in the present issue of *Neuron* (Bowers et al., 2004), in which they describe long-lasting upregulation of AGS3, a regulator of G protein activity, in specific brain regions following chronic cocaine administration. The family of membrane proteins known as G (guanine nucleotide binding) proteins along with their transmembrane GPCRs (G protein-coupled receptors) is one of the most widely used systems for signal transfer across neuronal plasma membranes, regulating the activity of numerous effector molecules within the cell. The binding of an extracellular ligand to its GPCR initiates a change in the conformation of GPCR subdomains such that the bound heterotrimeric G protein is released and in turn undergoes conformational change. Once unbound by the GPCR, the $G\alpha$ subunit exchanges GDP for GTP, triggering dissociation of the $\beta\gamma$ subunit from $G\alpha$. These events confer biological activity on the free $G\alpha$ subunit as well as the $\beta\gamma$ complex, both of which then regulate the functional activity of many downstream effectors, such as adenylate cyclases, cyclic nucleotides, intracellular protein kinases, and membrane ion channels. Recently, several families of proteins have been characterized that act as "accessory" G protein signal regulators, revealing novel mechanisms for controlling G protein signaling (Berman and Gilman, 1998; Blumer and Lanier, 2003). One family of modulators is termed the AGS proteins (activators of G protein signaling). One of these proteins, AGS3, complexes $Gi\alpha$ and thus diminishes signaling through $Gi\alpha$-mediated signal cascades.

Bowers et al. postulated that AGS3 within the prefrontal cortex might play a role in cocaine withdrawal and cocaine-induced neuroplasticity. In recent years, dysfunction in this brain region and its associated circuits has emerged as central to the process of addiction, particularly with regard to compulsive behavior, drug craving, and relapse (Jentsch and Taylor, 1999). Moreover, many studies have shown that chronic treatment with addictive drugs such as cocaine and opiates is associated with reduced $Gi\alpha$ protein-coupled receptor signaling in several brain areas (Nestler, 2001). Cocaine-induced behavioral sensitization, the phenomenon where previous cocaine treatment results in an enhanced response to the drug, also depends on PFC and its glutamatergic afferents. The authors treated rats with daily injections of cocaine for 1 week and then examined, during a withdrawal period, various aspects of AGS3 function in the prefrontal cortex and other brain regions known to play a role in addiction, such as nucleus accumbens, striatum, and ventral tegmental area. Levels of AGS3 were found to be strongly elevated in two of these regions, the PFC and nucleus accumbens core. Remarkably, whereas acute cocaine had no effect on AGS3 protein levels, the upregulation was found as late as 2 months after the end of treatment, suggesting a very enduring effect of chronic treatment, long after the drug is cleared. A similar profile was found in animals trained to self-administer the drug.

Bowers and colleagues then employed a clever method to mimic elevated levels of AGS3 in the prefrontal cortex. AGS3 contains a region with four G protein regulator (GPR) motifs in the carboxyl domain, where $Gi\alpha$ subunits can bind and be stabilized in the GDP bound conformation, preventing $Gi\alpha$ from binding GTP and subsequent inhibition of adenylate cyclase. This GPR peptide, when complexed with $Gi\alpha$ renders it unrecognizable by GPCR, effectors, or $\beta\gamma$ subunits. This peptide was then fused with a portion of the HIV-TAT protein, enabling transfer of the GPR peptide across the cell membrane (Schwarze et al., 1999). Thus, the TAT-GPR construct can be site-specifically microinjected and result in local temporary inactivation of $Gi\alpha$, much as endogenous AGS3 would work. A single point mutation in the GPR peptide abolishes $Gi\alpha$ binding and provides an inactive peptide (TAT-mGPR), serving as an

elegant control. After demonstrating that TAT-GPR reduced binding of [^{35}S]GTPγS (a nonhydrolizable, labeled analog of GTP) by Giα in vitro and that it had no observable neurotoxic effects in vivo, Bowers et al. microinjected TAT-GPR or TAT-mGPR into the PFC and gave rats an acute injection of cocaine. Animals that had received the active fusion peptide had markedly elevated levels of cocaine-induced motor activity, similar to what one would observe in rats that were "cocaine sensitized" (i.e., that had received multiple doses of cocaine). The cocaine sensitization-like phenotype was mimicked in other ways; for example, the rats had elevated levels of extracellular glutamate in the nucleus accumbens core as well as enhanced drug-seeking behavior when primed with a single injection of cocaine.

Not content with indirect evidence provided by the TAT-GPR studies, the authors then designed antisense oligonucleotides to directly reduce AGS3 expression in PFC. They showed that chronic cocaine treatment reduced dopamine D2 receptor-mediated Giα signaling in PFC tissue and that this reduction was restored by the AGS3 antisense treatment, showing that the increase in AGS3 was responsible for the reduction in Giα signaling. In a behavioral parallel, chronic infusion of the AGS3 antisense via minipumps in the PFC inhibited the expression of cocaine behavioral sensitization, an effect that was reversed when the minipumps were removed. Perhaps the most exciting finding of the paper is that the antisense treatment also completely prevented reinstatement of drug-seeking behavior induced by a priming cocaine injection. In rats trained to self-administer cocaine, lever pressing for cocaine delivery normally extinguishes when the cocaine is removed. Responding can be reinstated by a free cocaine injection, stress, or sensory cues previously associated with cocaine. This reinstatement of drug seeking behavior is a well-established animal model of relapse (Shaham et al., 2003). Here, Bowers et al. show that renewed interest in the drug (induced by the free cocaine injection) was blocked by selective and temporary reduction of AGS3. Again, when the antisense infusion was stopped, cocaine-induced reinstatement of drug seeking was restored. The involvement of AGS3 in drug reinstatement appears to be quite selective to cocaine-related reward, as infusion of the antisense into rats resuming lever pressing for food had no effect.

What are the implications of increased AGS3 activity in the PFC and one of its main targets, the nucleus accumbens core? Proteins regulating G protein function are conceptualized as "gatekeepers," poised at critical nodes of transmembrane signaling between receptors and effectors. Normally, intracellular events (such as the phosphorylation of many enzymes as well as alterations in gene transcription) are controlled by a delicate balance between the activity of stimulatory and inhibitory G proteins. If AGS3 is up in the drug withdrawal state (Figure 1), it would follow that signaling through receptors coupled to Giα would be decreased (e.g., D2, mu opiate, mGluR2,3), resulting in a shifted bias toward Gsα signaling (e.g., D1, β-adrenergic, CRF-R). For example, D1 signaling, intracellular activity and levels of adenylate cyclase, cAMP, and protein kinase A, and downstream targets such as CREB, and Fos-like proteins would be expected to be abnormally upregulated. There is abundant evidence for change in this direction in many models of addiction. Moreover, infusion of a D1 antagonist into the PFC blocks stress or cocaine priming reinstatement of cocaine-seeking behavior (Capriles et al., 2003). Thus, persistent increases in AGS3, long after drug exposure has ended, may be a key element in maintaining a shifted intracellular equilibrium despite long-term abstinence. Such a scenario provides a potential basis for the altered dopaminergic and glutamatergic transmission that is observed within the prefrontal-accumbens pathway with repeated cocaine use.

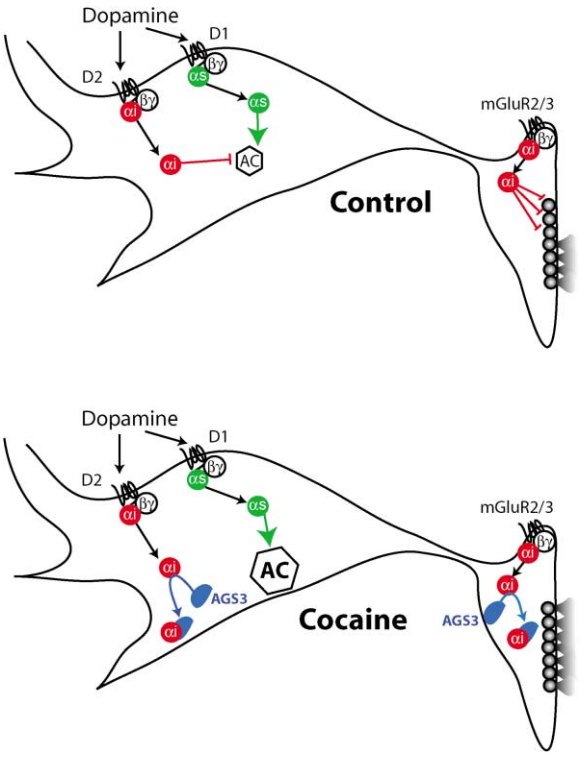

Figure 1. A Model of How Upregulated AGS3 Alters Prefrontal-Accumbens Neurotransmission in the Cocaine-Sensitized State

In control glutamatergic neurons projecting from the PFC to the accumbens core, a balance between D2 inhibition and D1 stimulation of adenylyl cyclase (AC) and presynaptic inhibition of glutamate release in the accumbens by mGluR2/3 is intact. Following withdrawal from repeated cocaine, AGS3 is elevated and inactivates Giα. The loss of Giα signaling causes the balance of dopamine receptor stimulation to bias toward D1 receptor activation of AC and inhibits the capacity of mGluR2/3 to regulate synaptic glutamate release. This dysregulation of glutamatergic cells results in the increased release of glutamate in the accumbens core that characterizes the reinstatement of drug seeking (courtesy of Peter Kalivas).

This elegant set of experiments provides new insight into the problem of relapse in drug addiction. Relapse can occur months or even years after cessation of drug taking and long periods of abstinence, suggesting that very stable, perhaps even permanent changes occur in the brain that may contribute to this vulnerability. Since the prefrontal cortex is critical for many cognitive functions involving inhibitory control, decision making, and emotional regulation, many have speculated that neuromolecular changes in this brain region may be central to the loss of control that accompanies advanced states of addiction. Several reports find that addicts have neuropsychological profiles indicative of prefrontal dysfunction, and neuroimaging studies show that cocaine addicts in protracted withdrawal have lowered resting metabolism

in the PFC (Goldstein and Volkow, 2002). However, *overactivation* of prefrontal regions is observed when addicts are presented with drug-associated cues, a phenomenon correlated with self-reports of increased craving (Bonson et al., 2002). In relapse, individuals fail to make a rational choice despite their former resolve and apparent knowledge of future adverse outcomes. Confronted by external cues that serve as "drug reminders," such individuals may experience conditioned autonomic responses and powerful cravings. If prefrontal cortical function is compromised by global cellular and molecular signaling abnormalities, the degree of voluntary control that the subject has over these feelings may be greatly impaired.

A number of questions are also raised by these findings. For example, how long lasting are these changes in the corticostriatal pathway? One week of one injection of cocaine per day is enough to induce a large increase in AGS3 3 and 8 weeks after the end of treatment. At 8 weeks, the PFC rise in AGS3 appears to be diminishing, but accumbens core levels remain just as elevated. While it is challenging to carry out very long-term studies, it is very important to assess such changes at much later time points. Additional key questions are which cells upregulate AGS3, pyramidal cells or interneurons or both, and what initial mechanism is responsible for AGS3 upregulation. It is curious that the authors did not observe the increase 1 week following cocaine but only at the later time points. Other mechanisms must account for the initially observed behavioral sensitization and reinstatement of drug seeking. It will be of interest to ascertain whether additional molecules within the Giα signaling complex are responsible for the early cocaine-sensitized phenotype and whether these candidates are linked to eventual persistent upregulation of AGS3. Furthermore, characterization of the precise molecular mechanisms underlying AGS3's modulation of prefrontal cortical circuits in cocaine sensitization, whether via excessive $\beta\gamma$ subunit modulation of membrane conductance and/or via indirect modulation of downstream targets of Giα influencing neuronal excitability, is needed. Another key question is whether upregulated AGS3 is specific for reinstatement to cocaine-seeking behavior or rather is a common mechanism shared by exposure to all addictive drugs.

Integration of methodologies from disparate disciplines such as biochemistry, molecular biology, and behavioral neuroscience enables a powerful approach for investigating complex problems such as addiction. Bowers and colleagues have employed this strategy and elucidated a putative novel target for pharmacotherapies in the treatment of addiction. This study is a fine example of the kind of interdisciplinary research that is essential for the development of mechanism-based pharmacological, cognitive, and behavioral therapies for neurobehavioral disorders.

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Hippocampal Place Cells Demand Attention

Hippocampal representations of the environment are thought to play a fundamental role in the encoding, storage, and retrieval of declarative memory. In this issue of *Neuron*, Kentros and coworkers show that new hippocampal representations stabilize only when animals are attentive.

Evidence from many sources implicates the hippocampus as a brain area of particular importance for the fast encoding and storage of memory. Hippocampal memories are expressed at the neuronal level as representations reflecting the structure of the external environment. One prominent correlate of hippocampal pyramidal cells is their location-specific firing (O'Keefe and Nadel, 1978). The firing of a hippocampal place cell is influenced by sensory information reaching the hippocampus from adjacent parahippocampal areas but may also signal information stored in memory. The latter is particularly evident during conditions of sensory deprivation, as when room lights are turned off (Quirk et al., 1990) or when dominant landmarks are removed (O'Keefe and Conway, 1978). Under such conditions, the patterns of discharge often remain similar to those that pervaded on preceding trials with the uncorrupted input, suggesting that their firing expresses memory.

In agreement with the view that place fields are not driven only by sensory inputs, numerous reports suggest that the development of a particular hippocampal spatial representation depends on task requirements. Minor changes in sensory input may give rise to totally different spatial maps (Bostock et al., 1991), and new