

Rewarding withdrawal

In an exciting study, **Dr Youssef Sari** is investigating approaches to reverse the impulse to alcoholism or drug dependency by augmenting the brain's capacity to maintain normal neurotransmitter levels and function



Could you first outline your project goals?

The prefrontal cortex (PFC) in the brain is the moderator for decision making. In addicts, the PFC is very active and releases – in particular and at the slightest provocation – a neurotransmitter called glutamate. We aim to investigate the role of the glutamatergic system in the central reward regions of the brain that are involved in alcohol dependence, and ultimately seek to develop drugs that can successfully treat alcohol addiction.

What evidence is there to suggest that many aspects of alcohol and drug dependence involve changes in glutamate transmission?

Neuroadaptations of the glutamatergic system play a key role in alcohol tolerance, dependence and withdrawal. The selective effects of alcohol include inhibition of glutamatergic neurotransmission by alteration of N-methyl-D-aspartate (NMDA) receptors. One of the effects of chronic alcohol exposure is the up-regulation of NMDA receptors that are part of the compensatory mechanism, which results from chronic inhibition of glutamatergic neurotransmission. In addition, the effects of alcohol withdrawal are associated with increased extracellular glutamate levels in the striatum of alcohol-dependent rats and enhanced NMDA sensitivity in the nucleus accumbens (NAc). Importantly, a number of

studies have reported that alcohol also alters glutamate transport.

Although the neurocircuitry of the glutamatergic system is not fully defined, it has been suggested that this system within the PFC and the NAc plays a critical role in drug reinforcement. Both regions receive substantial input from midbrain dopaminergic neurons, and all major drugs of abuse, including alcohol, increase forebrain dopamine transmission.

Glutamatergic projections from the PFC to the NAc are also critical in the expression of addictive behaviors. Therefore, our studies focus on the analysis of the function of a protein called glutamate transporter-1 (GLT1) in these two key brain reward regions.

Can you explain the role of GLT1 in developing therapeutics for the treatment of alcohol abuse and dependence?

We have found that elevation of the expression of GLT1 was associated with a reduction in alcohol consumption and attenuation of relapse to alcohol drinking behavior in rats. GLT1 might therefore be considered as a potential therapeutic target for the treatment of alcohol abuse and dependence.

Other researchers have tested more than 1,040 approved drugs to determine those which might be effective at removing glutamate from the space between neurons. It turns out that a β -lactam antibiotic – ceftriaxone – increases the expression of GLT1 and decreases the amount of extracellular glutamate available to activate addictive behaviors. This drug has been used to treat meningitis and is in phase III clinical trial for the treatment of amyotrophic lateral sclerosis (ALS).

What did you discover during your examination of alcohol-preferring (P) rats?

P rats naturally prefer drinking alcohol to plain water and so we used this model to measure the effectiveness of ceftriaxone in reducing alcohol cravings and consumption. After five weeks of a constant free choice of

an alcoholic beverage, the rats developed alcohol dependence. We administered ceftriaxone to the rats every day for five days and measured their alcohol consumption. After treatment with ceftriaxone, the rats consumed significantly less alcohol. This reduction was associated with increased levels of expression of GLT1 in brain reward regions, including the PFC and NAc.

We have also tested another drug, GPI-1046, that has shown to be effective in reducing alcohol consumption associated with elevation of GLT1 levels.

Could you describe the mechanisms of adenosine and glutamate signalling in neuron-glia interactions?

Adenosine plays an important role in regulating the activity of neurons and controlling neurotransmitters, including gamma-aminobutyric acid (GABA), glutamate and dopamine. Alcohol has been shown to increase extracellular adenosine levels, which in turn regulates the ataxic and hypnotic/sedative effects of alcohol. Adenosine signalling is also involved in the homeostasis of major inhibitory (GABA) and excitatory (glutamate) neurotransmission through neuron-glia interactions. These interactive mechanisms regulate the effect of alcohol and sleep. Furthermore, adenosine exerts its function through several adenosine receptors and regulates glutamate levels in the brain, which modulate alcohol dependence and sleep patterns.

What will be the next steps for your research?

Now that we have shown the effectiveness of ceftriaxone in reducing addictive behaviors, we are working to create synthetic analogues. Ceftriaxone is an antibiotic and it would be advantageous to have therapeutic compounds that do not have the antibiotic form. There are other compounds that activate GLT1. We are currently investigating the signalling pathways involved in the mechanism of action of these drugs.

Alcohol dependency: the sobering reality



Studies at the **University of Toledo** have identified that the compulsion to indulge in harmful behaviors can be modulated by adjusting the expression of fundamental neuron-glia messaging proteins in the brain – research that could have important implications for alcoholism

ALCOHOLISM IS WIDELY recognised as a disease rather than a lifestyle choice – a perception first put forward by the Alcoholics Anonymous organisation in the 1930s. The perception that alcoholism is a physical disease rests on the evidence that, despite widespread knowledge of the damaging effects of excessive drinking on health – from life-threatening liver disease to impaired brain function – the nature of an alcoholic's addiction is such that, presented with the opportunity to drink, he/she will most often succumb. Emerging evidence suggests that physiological changes are responsible for the formation of addiction to and dependency on alcohol and other drugs, though such changes do not arise from disease (Krystal et al., 2003). A key source of dependency is thought to be alcohol-induced changes to the transport of glutamate, an amino acid that is responsible for messaging between neurons in the brain (Smith, 1997).

Dr Youssef Sari, Assistant Professor of Pharmacology at the University of Toledo in Ohio, USA, is exploring the role of the main glutamate transporter, glutamate transporter 1 (GLT1) – an integral membrane protein – in alcohol dependency. The role of GLT1 is to clear glutamate from clefts between synapses by taking it up and sequestering it. Sari's hypothesis is that normal glutamatergic transmission in central reward regions of the brain thus modulates the intake of alcohol or drugs such as cocaine, and that perturbations of this system result in substance abuse and dependency.

Dysfunction in the glutamatergic excitatory system is known to be involved in neurodegenerative diseases. As there are parallels between the effects of alcohol on the brain and some neurodegenerative diseases such as Huntington's disease, Sari proposes

that the alcohol causes GLT1 to fail to properly fulfil its role. This proposal has been validated in several recent projects in Sari's laboratory, in which he has determined that treatment of addicted rats with an antibiotic – ceftriaxone, which elevates GLT1 levels (Rothstein et al., 2005) – causes a significant reduction in alcohol consumption, and inhibits relapse into cocaine or alcohol dependency behaviors (Sari et al., 2009; Sari et al., 2011; Qrunfleh et al., 2013).

THE NATURE OF DEPENDENCY

The burden of alcohol dependency is massive. It afflicts nearly 14 million people and is responsible for around 79,000 deaths annually in the US alone. On a global scale, the World Health Organization (WHO) attributes 2.5 million deaths annually to the disease: "Alcohol dependency is defined by four characteristics: craving, or the strong need to drink; loss of control, or not being able to stop; dependence, or having withdrawal symptoms such as seizures, nausea and sweating after stopping; and tolerance, or requiring greater quantities to obtain the same effect," Sari explains.

The prefrontal cortex (PFC) and nucleus accumbens (NAc) regions in the brain receive substantial input from midbrain dopamine neurons, and all major drugs of abuse, including cocaine, are known to increase forebrain dopamine transmission (Kalivas, 2004). However, dopamine involvement in relapses into drug abuse relies on an increase in glutamate, the primary driver of PFC neurons. "The importance of these glutamatergic projections from the PFC to the NAc and the ventral tegmental area have been observed in neuroimaging studies performed during craving periods in several different paradigms, for commonly abused drugs such

as alcohol, cocaine, methamphetamine, heroin and nicotine (Goldstein and Volkow, 2002)," Sari elaborates.

In 2009, Sari tested the hypothesis that, since an increase in glutamate transmission triggers dopamine to engender relapse behavior, the elevation of GLT1 levels in the PFC and NAc regions of the brain should prevent relapse into cocaine use by reducing the extracellular glutamate levels. Indeed, Sari and colleagues found that ceftriaxone treatment attenuates relapse to cocaine seeking behavior (Sari et al., 2009).

Though the neurochemistry is different in alcohol and cocaine addiction, glutamate plays a similar role in both cases. Sari deduced that the

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Economic burden of sleep disorders related to alcohol dependency in the US alone



Key Publications

findings from his study with cocaine-addicted rats provided a solid foundation for targeting GLT1 in alcohol dependency. In recent projects, Sari's lab has examined the effects of ceftriaxone and another drug, GPI-1046, that raises GLT1 levels in alcohol-preferring (P) rats (Sari et al., 2011; Sari and Sreemantula, 2012): "Increasing the expression of GLT1 in the brain of a rat exposed to alcohol reduced the levels of extracellular glutamate available to activate neurons in central reward regions of the brain, and thus decreased the craving initiated by it," he observes.

ADJUSTING THE EFFECTS OF ALCOHOL

In a further review of the implication of adenosine and glutamate in alcoholism and sleep disorders, Sari and his collaborators discussed the importance of neuron-glia signalling between glutamate and adenosine, which plays a critical role in proper brain function and regulates the effects of alcohol on coordination and wakefulness (Nam et al., 2012). Alcohol increases extracellular adenosine levels, which decreases glutamate neurotransmission; equally, glutamate increases the release of adenosine.

Importantly, the team reported that alteration of glial transporters, GLT1 and equilibrative nucleoside transporter 1 (ENT1) occurred with exposure to alcohol (Sari et al., 2013 in press). Researchers found that astrocyte-dependent purinergic signalling additionally appeared to have an impact on dependent behavior and Sari feels that this needs further clarification. However, Ceftriaxone administration restored GLT1 and ENT1 levels to normal and Sari and his team found that the risk of excessive glutamate clearing was low.

The team has established that ceftriaxone administration in P rats is long-lasting, being effective after five weeks of alcohol drinking and lasting up to 10 days post-treatment (Sari et al., 2011). They have also ascertained that ceftriaxone administration can stop the rats from relapsing into drinking alcohol, but that it does not interfere with less harmful compulsions (Qrunfleh et al., 2013). To Sari, therefore, proper glutamate transport provides protection against deleterious addictions and targeting GLT1 expression is clearly the way forward: "A focus on the glutamatergic system as a prime candidate for mediating drug and alcohol dependence is the key to paving the path for new treatment of such addictions," he forecasts.

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INTELLIGENCE

THE ROLE OF GLT1 IN THE MODULATION OF ALCOHOL-DRINKING BEHAVIOR IN P RATS

OBJECTIVES

To investigate the potential therapeutic role of glutamate transporter 1 (GLT1) on alcohol-drinking behavior with a genetically selected high alcohol-consuming rat line: the alcohol-preferring (P) rat.

KEY COLLABORATORS

Dr Doo-Sup Choi, Mayo Clinic, Rochester

Dr James Leighton, Columbia University, New York

Dr George Rebec, Indiana University

Dr Bryan Yamamoto, University of Toledo

Dr Richard Bell, Indiana University

Dr Paul Rosenberg, Harvard Medical School

Dr Jeffery Rothstein, Johns Hopkins University

FUNDING

National Institutes of Health (Grant no. R01AA019458).

CONTACT

Dr Youssef Sari

Assistant Professor of Pharmacology

University of Toledo
College of Pharmacy
& Pharmaceutical Sciences
Department of Pharmacology
Health Science Campus, Mail Stop 1015
3000 Arlington Avenue, HEB283C
Toledo, OH 43614, USA

T +1 419 383 1507
E youssef.sari@utoledo.edu

DR YOUSSEF SARI received his doctorate in neuroscience from Pierre and Marie Curie University, Institute of Neuroscience (Paris, France) and subsequently held postdoc and faculty research positions at Indiana University. Sari holds a faculty position at the level of Assistant Professor of Pharmacology at the University of Toledo, College of Pharmacy and Pharmaceutical Sciences, Toledo, Ohio, USA. His research focuses on the role of GLT1 in alcohol-drinking behavior using compounds targeting this transporter. Sari is also testing compounds for the treatment of diseases involving oxidative stress.



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