Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Special Issue: *Addiction Reviews*
COMMENTARY

The neurobiology of addiction

George R. Uhl,[1] George F. Koob,[2] and Jennifer Cable[3]

[1]New Mexico VA Healthcare System, Albuquerque, New Mexico. [2]NIH/NIAAA, Bethesda, Maryland. [3]jennlcable@gmail.com

Address for correspondence: George Uhl, New Mexico VA Healthcare System, Albuquerque, NM 87108. George.Uhl@va.gov

Substance and alcohol use disorders impose large health and economic burdens on individuals, families, communities, and society. Neither prevention nor treatment efforts are effective in all individuals. Results are often modest. Advances in neuroscience and addiction research have helped to describe the neurobiological changes that occur when a person transitions from recreational substance use to a substance use disorder or addiction. Understanding both the drivers and consequences of substance use in vulnerable populations, including those whose brains are still maturing, has revealed behavioral and biological characteristics that can increase risks of addiction. These findings are particularly timely, as law- and policymakers are tasked to reverse the ongoing opioid epidemic, as more states legalize marijuana, as new products including electronic cigarettes and newly designed abused substances enter the legal and illegal markets, and as "deaths of despair" from alcohol and drug misuse continue.

Keywords: addiction; substance abuse; neuroplasticity; alcohol; marijuana; nicotine; opioids

Addiction neurobiology is superbly situated to benefit from many neuroscience advances. Advanced imaging that reflects neuronal activity and neurochemistry in humans and experimental animals provides substantial insights into meso-scale brain changes that are highly relevant for addictions. Addiction researchers' early adoption of optogenetic and chemogenetic approaches has provided elegant support for and refinement of hypotheses about roles for specific circuits in addiction-related behaviors and physiology.

Much progress in the neurobiology of addiction can be placed into a heuristic three-stage addiction cycle framework: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. This framework is supported by multiple neuroadaptations in three corresponding domains: (1) increased incentive salience, (2) decreased brain reward and increased stress, and (3) compromised executive function; and in three major neurocircuits: basal ganglia, extended amygdala, and prefrontal cortex (Fig. 1). The focus in the neurobiology of addiction has changed with emphasis on the mechanisms of acute reward in the binge/intoxication stage broadened to include neuroadaptations that are consequent to drug exposure. These include mechanisms driving incentive salience, compulsive habits, deficits in reward and recruitment of stress during the withdrawal/negative affect stage, and modulation of executive function systems and mnemonic systems (and being modulated by mnemonic processes) in the preoccupation/anticipation stages of substance use disorders.

Addiction science is also well poised to use results from a number of the changes in the addictions landscape.[a] Legalization of cannabis use by states provides opportunities to examine effects of reduced penalties for cannabis production and use; neighboring states that do not legalize provide

[a]Both the size and the pace of change in the burdens that addictive substances and substance use disorders place on individuals, families, communities, and nations are undisputed. Also worth noting are certain changes over time of perspectives on addiction science. Language can be confusing in this area. For example, the American Psychiatric Association/Diagnostic and Statistical Manual (DSM) diagnoses have changed over time: current *substance use disorders* were previously *substance abuse and substance dependence. Dependence*, in turn, encompassed both physical dependence manifested by withdrawal syndromes and psychological dependence noted in older diagnostic systems.

doi: 10.1111/nyas.13989

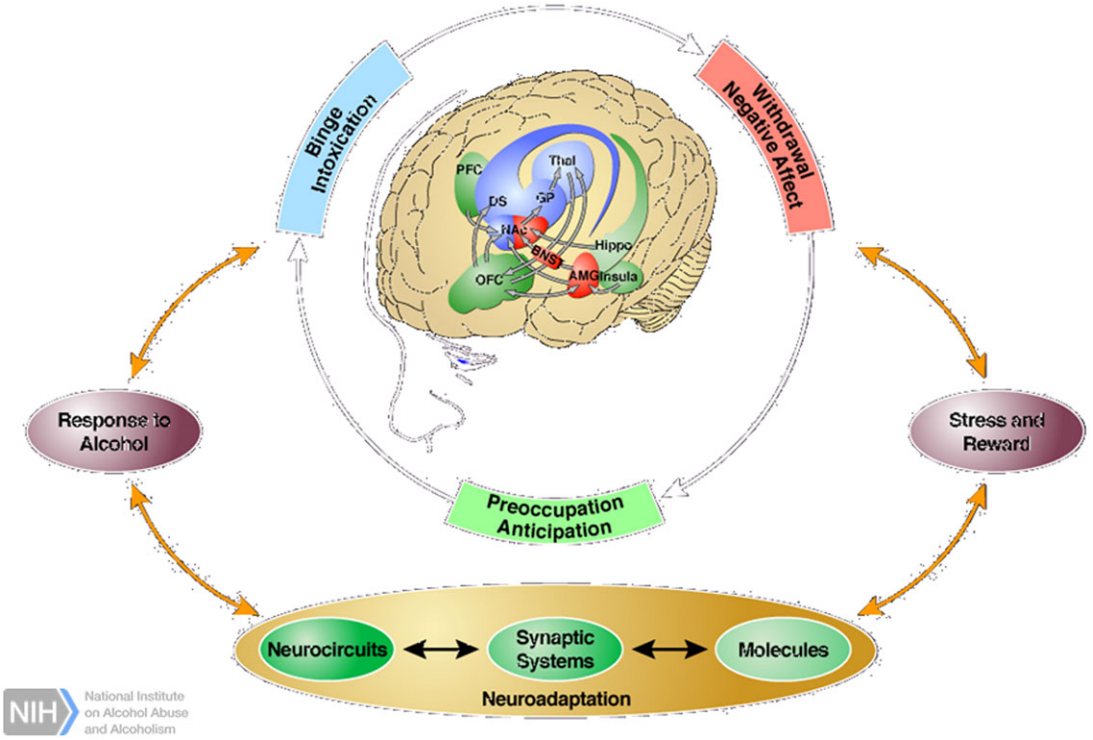

Figure 1. Conceptual framework for neurobiological bases of the transition to substance use disorders. PFC, prefrontal cortex; DS, dorsal striatum; GP, globus pallidus; NAc, nucleus accumbens; Hippo, hippocampus; Thal, thalamus; BNST, bed nucleus of the stria terminalis; AMG, amygdala; OFC, orbitofrontal cortex. Reproduced with permission from *Neuropsychopharmacology*.[222]

control environments. Restrictions on opioid prescribing now provide opportunities to examine how reduced availability of pharmaceutically prepared opioids influences patterns of distribution and the use of illicitly prepared opioids, as well as the treatment of pain for which pharmaceutical opioids were prescribed. Illicit designer substances provide challenges in understanding the actions of novel pharmacological products in humans even before laboratory animal and *in vitro* testing. Differences, by region and over time, in availability of behavioral and pharmacological addiction therapeutics provide opportunities to assess their worth in new ways.

By definition, drugs form a vibrant part of the neuropharmacology of addiction. Since addictive substances themselves are central to addiction pathogenesis, our etiologic understanding of addictions can advance at greater rates than in the neurobiology of brain illnesses whose etiologic agents are less well understood.

Addictions have usurpation of motivation at their cores. Many have underlined the ways in which substance use, established by rewarding processes, can be maintained by altering motivation, including driving incentive salience, establishing compulsive-like habits, engaging negative reinforcement, and facilitating impulsivity.

In 2017, 19.7 million people age 12 or older in the United States were estimated to have a substance abuse disorder related to alcohol or illicit drug use. This value includes 14.5 million people with an alcohol use disorder (AUD) and 7.5 million people with an illicit drug use disorder, the most common illicit drug being marijuana. Tobacco use also remains prevalent, with 48.7 million current cigarette smokers, of whom 27.8 million smoke daily, and 11.4 million smoke at least a pack per day.[1] Substance abuse disorders exert not only a significant public health burden—individuals with substance use disorders are more likely to suffer from chronic pain, hypertension, injuries, poisonings, and overdose[2]—but they also impose significant economic burdens. Costs associated with substance abuse disorder exceed US$700 billion annually due to crime, lost

Ann. N.Y. Acad. Sci. 1451 (2019) 5–28 © 2019 The Authors. *Annals of the New York Academy of Sciences* published by Wiley Periodicals, Inc. on behalf of The New York Academy of Sciences.

work productivity, and health care;[3] US$250 billion due to alcohol; and US$300 billion due to tobacco. Adolescents and young adults are particularly at risk for developing substance use disorders; areas of their brains responsible for evaluating risk, weighing consequences, and making decisions are not fully developed until the mid-20-year-old age range.[4] Individuals who begin using illicit addictive substances earlier in life ultimately consume more addictive substances more frequently and have higher rates of substance use disorders.[5]

While preventive and treatment strategies can reduce substance use and substance use disorders, effects of available prevention and treatment strategies are often modest and short term. New research is elucidating the neurobiological changes, genetic markers, and epigenetic changes associated with addictions. These developments are identifying new targets for treatments and should facilitate personalized/tailored preventive and treatment approaches to maximize effectiveness. However, advances in our understanding of addiction biology can only provide benefit if they are adopted by law and policymakers as evidence-based policies and programs.

In May 2016, the Aspen Brain Forum and the New York Academy of Sciences brought together leaders in neuroscience, addiction medicine, drug and alcohol abuse, and science advocacy and policy to discuss and update topics in the neurobiology of addiction at the 2.5-day conference "The Addicted Brain and New Treatment Frontiers: Sixth Annual Aspen Brain Forum." The conference supported the mission of the Aspen Brain Forum to produce, host, and fund an annual meeting on innovative topics in neuroscience to advance global collaborations and scientific breakthroughs. The meeting also highlighted substantial progress and challenges in addictions and in the neuroscience of understanding these substance use disorders. The Aspen Brain Forum neurobiology work also reflects the striking intersections between policy and science. In few other fields would the contributions of such a talented and tireless advocate for addiction and mental health as Patrick Kennedy (see below) seem so appropriate and natural for a "neurobiology" program.

This report presents a synthetic report of individual presentations at the Sixth Annual Aspen Brain Forum and ends with some reflections on the current state of the field.

The role of dopamine in addiction

Addictive drugs are inherently rewarding. They highjack the brain's dopamine system to increase dopamine levels in the nucleus accumbens, a key focal point for reward neurocircuitry in the brain.[6] While dopamine is critical for the rewarding effects of drugs, its role in substance use disorders is still evolving. Nearly 20 years ago, Nora Volkow (National Institute on Drug Abuse, National Institutes of Health) showed via positron emission tomography imaging that higher dopamine levels correspond with a more intense high in healthy volunteers given intravenous methylphenidate (MPH), a central nervous stimulant also known as Ritalin. There was considerable variability in dopamine levels across subjects; some individuals experienced neither increased dopamine levels nor "high." Administration of oral MPH, which takes longer to enter the brain, resulted in no high with slower increases in dopamine levels.[7]

Since the rate of dopamine increase plays a factor in whether a drug will produce a rewarding effect, the different properties and effects of dopamine receptors in the brain are likely to play significant roles. The prefrontal cortex contains both dopamine D1 and D2 receptors. D2 receptors have an approximately 10- to 100-fold greater affinity for dopamine than D1 receptors and are therefore activated at lower dopamine concentrations. Under normal circumstances, the prefrontal cortex receives a low level, stable flow of dopamine owing to relatively slow, tonic firing of dopamine neurons in the ventral tegmental area (VTA) that project to the cortex. However, in response to an unexpected event, such as an extraordinary reward or very aversive event, dopamine neurons fire much more quickly. This phasic firing results in an abrupt, yet transient, increase in dopamine. The high levels of dopamine achieved during phasic firing are able to activate D1 receptors and are thought to be required for dopamine's full rewarding effects.[8,9] Drugs of abuse, particularly psychostimulants, mimic the high dopamine concentrations produced by phasic firing and thus activate both D1 and D2 receptors.[10]

D1 receptors stimulate both reward, via pathways modulating the striatum and cortex, and conditioning and memory mechanisms that involve the amygdala, medial orbitofrontal cortex (OFC), and hippocampus. The conditioning/memory processes critical to addiction allows individuals to

automatically associate a stimulus with a reward or punishment. Perhaps paradoxically, several studies have shown that addictive drugs fail to increase dopamine release in addicted individuals compared with nonaddicted controls. MPH did not significantly increase dopamine levels among active[11] or detoxified cocaine addicts.[12] Cocaine users also reported less of a high from MPH than controls.[12] However, among active addicts shown a video to produce craving, increased dopamine was observed in the dorsal striatum. The magnitude of this dopamine increase was associated with the extent of drug craving.[11] These data suggest that in addiction there is thus a switch from the drug itself initiating dopamine release to drug cues and stimuli initiating dopamine release. This shift from reward to conditioning involves dopamine phasic firing leading to drug cravings and compulsive drug use in response to drug and other conditioned cues.[6]

Normally, D2 receptors modulate the effects of D1 receptors via the striatal indirect pathway;[10] however, several studies have shown that addicted subjects have lower expression of dopamine D2 receptors.[13] Reductions in D2 receptors among addicted subjects are associated with decreased activity in the OFC, anterior cingulate gyrus, and dorsolateral prefrontal cortex areas of the brain involved in emotion regulation and decision making. Because impairments in the orbitofrontal and anterior cingulate cortices are associated with compulsive behaviors, impaired dopamine signaling in these areas in addicted subjects may be partially responsible for their compulsive behavior and impulsivity.[6] In animal studies, increased dopamine D2 receptor expression in the nucleus accumbens reduced drug consumption in models of both alcohol and cocaine dependence.[14,15] In humans, a recent study in methamphetamine users demonstrated that regular aerobic exercise can upregulate striatal dopamine D2 and D3 receptors; whether this results in reduced cravings and drug use remains to be seen.[16]

Computational modeling of dopamine cells

According to a computational model of dopamine release in response to rewards and expectations, dopamine neurons encode reward prediction errors in their firing rates—they increase their firing rates if results are better than expected and decrease their firing rates if results are worse than expected.[17] To test this model and its relationship to behavior, P. Read Montague (Virginia Polytechnic Institute and State University; University College London) has used functional magnetic resonance imaging (fMRI) to monitor the effects of reward prediction error on both brain activity and future behavior in subjects participating in a betting task in a fictitious market. The study found neural signatures associated with reward prediction error and fictive error (how much a person gains versus how much they could have gained if they had bet more). Fictive error was associated with activation in the ventral caudate, ventral putamen, and posterior parietal cortex as well as with behavioral changes. The higher the fictive error, the more likely a person was to change their next bet.[18] Fictive error signatures were present in the brains of both smokers and nonsmokers; the magnitude of these signatures did not correlate with a change in behavior in smokers.[19]

Using electrochemistry to monitor dopamine release in real time while subjects completed such tasks showed that dopamine release roughly correlates with market activity at long timescales. However, at short, millisecond timescales, a different pattern emerged. With high bets, increases in dopamine fluctuations correlated with reward prediction errors; however, as the bet size decreased, the correlation reversed. At low bet sizes, increased dopamine fluctuations were seen with negative errors and vice versa. This behavior suggests two sources of dopamine fluctuation—one that communicates a prediction error and one that communicates a fictive error.[20]

Endocannabinoids and addiction

We have come a long way in understanding the endocannabinoid system and the potential for therapeutic interventions directed at this system, according to Susan Weiss (National Institute on Drug Abuse). Tetrahydrocannabinol (THC) and cannabidiol mimic aspects of the effects of the endogenous cannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Under endogenous conditions, AEA and 2-AG are released into the synapse by postsynaptic neurons. They bind to cannabinoid receptors on presynaptic neurons to dampen their activity, thus participating in a negative feedback loop. The functional effects observed depend significantly on which neural circuits are involved.[21]

Ann. N.Y. Acad. Sci. 1451 (2019) 5–28 © 2019 The Authors. *Annals of the New York Academy of Sciences* published by Wiley Periodicals, Inc. on behalf of The New York Academy of Sciences.

However, much less is known about therapeutic effects of cannabis itself. Part of the complexity stems from the fact that marijuana contains myriad chemicals—hundreds of cannabinoids as well as other chemicals that differ in concentration depending on strain. THC and cannabidiol are currently the two most studied components of marijuana. These two chemicals have profoundly different effects on the brain, with THC producing the high associated with marijuana use. Exogenous administration of cannabinoids is one therapeutic strategy to target the endocannabinoid system. Sativex® (nabiximols), a combination of THC and cannabidiol, has been approved in Europe for spasticity associated with multiple sclerosis[22] and was granted fast track designation by the U.S. Food and Drug Administration (FDA) for the treatment of pain in patients with advanced cancer.[23] Cannabidiol is being investigated for its anticonvulsant properties.[24]

Other druggable targets in the endocannabinoid system may offer opportunities for different, even more precise, modulation of these systems. For example, fatty acid amide hydrolase (FAAH) inhibitors, which block the breakdown of AEA, might be expected to have greater effects on activated circuits. In animals and humans, FAAH inhibitors have been shown to reduce anxiety- and depression-like behaviors, to enhance social behavior in autism spectrum disorders, and to reduce nicotine addiction.[25–28]

Several studies have also found associations between frequent cannabis use and the risk of psychosis.[29–31] Many questions remain that will help to better understand this connection, including the effect of cannabis on neurodevelopment as well as to better understand the reasons that people with psychotic disorders continue to use cannabis if it exacerbates their psychoses.

Learning mechanisms underlying addiction: goal directed versus habitual behavior

Initially, drug taking begins as a voluntary, goal-directed behavior. People take drugs because they are seeking a specific high or reward. However, in some people, the behavior becomes compulsive and is no longer associated with seeking a reward. This change is associated with a shift in circuitry within the brain. While structures like the basolateral amygdala and nucleus accumbens are necessary to acquire prolonged drug-seeking behavior,[32,33] they become less important after the behavior has been established. Then, the dorsolateral striatum plays a more important role.[34–36] In animals engaged in long-term drug-seeking behavior, a large increase in dopamine is observed in the dorsal striatum, but not in the nucleus accumbens core or shell.[33,37] Blocking dopamine receptors in the dorsal striatum, but not the nucleus accumbens core, reduced well-established, habitual drug-seeking behavior.[38]

Barry Everitt (Cambridge University) and David Lovinger (National Institute on Alcohol Abuse and Alcoholism) are exploring what changes occur in the brain when an animal shifts from goal directed to more habitual behavior. One way to monitor the habitual nature of behavior uses a devaluation training scheme, allowing the animal to eat to satiety or making them ill after feeding. In a classic 1981 experiment, Adams and Dickinson showed that among animals trained to press a lever to receive food, those trained for short times stopped seeking food after satiety, demonstrating goal-directed behavior. However, animals trained for a long time continued to seek food even after satiety, indicating habitual behavior.[39]

Everitt described a procedure that devalues cocaine in which animals must first engage a drug-seeking lever to get access to a drug-taking lever. Pressing on the drug-taking lever results in an infusion of cocaine. To devalue the drug, the seeking lever and cocaine are removed for a period of time, leaving only the drug-taking lever. Afterward, the seeking lever is reintroduced. If the animal's behavior is goal directed, they will not engage the seeking lever; however, if the behavior is habitual and compulsive, they will engage the seeking lever, despite devaluation. In animals trained for a short time to seek cocaine, devaluation reduced drug-seeking behavior and did not promote habitual behavior. However, devaluation had no effect in animals trained to seek cocaine over a long time.[40–42] Inactivating the dorsolateral striatum in animals with habitual behavior restored the effects of devaluation.[40] Similar results have been seen for alcohol[43,44] and nicotine.[45]

When behaviors switch from goal directed to habitual, there is a corresponding switch from the ventral to dorsal striatum. Disconnecting the ventral and dorsal striata impaired cocaine-seeking behavior in animals, suggesting that there is a

connection between the two systems.[46] Animal studies also suggest a functional link between the dorsolateral striatum and the basolateral amygdala, which processes environmental cues that trigger habitual behaviors.[47]

Lovinger uses a different training scheme to monitor habitual versus goal-directed behavior in mice trained to press a lever to receive food. The mice are subjected to two training paradigms that alter the value of food, one that fosters goal-directed behavior, and another that fosters habitual behavior. Using these techniques, he can examine different behaviors within the same animal and observe the effects of manipulating neural circuits on behavior.

Lovinger showed that the connection between the OFC and dorsomedial striatum (DMS) is critical for goal-directed behavior. Introducing lesions into the OFC fostered habitual behavior[48] as did inhibiting OFC projection neurons and OFC synapses with the chemogenetic tools using the DREADD procedure.[49] Conversely, increasing OFC firing and OFC to DMS input using activation via channelrhodopsin (ChR2) increased habitual behaviors in his model.

The brain has several mechanisms to modulate the OFC/DMS synapses and either promote or inhibit habit formation. Several receptors found at the presynaptic terminus, including the endocannabinoid CB1 receptor, can suppress signaling to promote habitual behavior. Conditional knockout of CB1 in the OFC in mice resulted in strong goal directedness and interference with the ability to form habits. These results were recapitulated when CB1 was knocked out only in OFC neurons that project into the DMS.[50]

The data suggest that the OFC to DMS pathway is important for the shift from goal directed to habitual behavior. If these pathways are strong, goal-directed behavior is favored. As these pathways are suppressed over more and more learning trials during the natural learning process, via receptors such as CB1, behavior becomes more habitual.

The dark side of addiction: stress neurocircuitry/mechanisms underlying addiction

The role of corticotropin releasing factor and dynorphin in the dark side of addiction

The brain's stress and reward systems are intricately linked. Moderate forms of stress, such as skydiving, can also activate the reward system. Excessive activation of the reward system, as in the case of excessive drug use, can also engage the brain's stress system. As individuals who have become dependent on drugs lose normal function of aspects of their reward systems, they can gain activation of their stress system as well.

George Koob (National Institute on Alcohol Abuse and Alcoholism) described his longstanding fascination with understanding the connections between stress and addiction and how they contribute to a powerful additional source of motivation in addiction: negative reinforcement. Here, Koob argues that the driving force for negative reinforcement (where removal of an aversive stimulus, drug withdrawal, increases the probability of drug seeking and taking) is the negative emotional state of withdrawal mediated by stress-related neurotransmitters, particularly corticotropin-releasing factor (CRF) and dynorphin. He emphasized that there are also many other stress-related neurotransmitters up- or downregulated in addiction that warrant further study.[51,52]

During acute stress, the peptide CRF is activated in the extended amygdala during withdrawal from abused substances that include alcohol,[53] cocaine,[54] cannabinoids,[55] opioids,[56] and nicotine.[57] CRF antagonists decrease withdrawal-induced anxiety-like responses in animals,[58–60] decrease the escalation associated with extended access to drugs of abuse, and decrease alcohol intake in alcohol-dependent rats while having no effect on alcohol intake in nondependent rats.[61]

These dynamic changes in extrahypothalamic CRF may begin with the initial hormonal response of increased release of glucocorticoids driven by hypothalamic CRF. However, during periods of chronic stress, high levels of glucocorticoids decrease CRF levels in the hypothalamic periventricular nucleus while increasing CRF levels in the amygdala.[62] A similar effect has been seen in drug-dependent animals.[63] Compulsive-like drug taking thus increases CRF levels in the amygdala, prefrontal cortex, and VTA, contributing to stress-like responses and negative emotional states, which provide the motivation for sustaining compulsive-like drug taking via negative reinforcement. Similar to CRF antagonists, glucocorticoid antagonists reduced alcohol consumption in alcohol-dependent animals, but not in nondependent

controls.[64] A recent human laboratory study in nontreatment-seeking individuals with alcohol addiction demonstrated that the glucocorticoid antagonist mifepristone reduced craving and drinking compared with placebo.[65]

Dynorphin is a kappa opioid whose expression can be modulated by activation of dopamine or opioid receptors.[66] Unlike other opioids, kappa opioids induce feelings of dysphoria. Compulsive drug taking increases dynorphin levels in the nucleus accumbens and amygdala, contributing to a dysphoric-like state. High levels of dynorphin signal through a negative feedback loop to turn off dopamine production, and kappa opioid agonists decrease extracellular dopamine levels in the nucleus accumbens.[67] The kappa antagonist nor-binaltorphimine (nor-BNI) decreases excessive drinking in alcohol-dependent rats while having no effect in nondependent animals, similar to CRF and glucocorticoid antagonists.[68] Injecting nor-BNI into dynorphin-expressing areas of the nucleus accumbens blocks withdrawal-induced increases in alcohol administration in rats.[69] Thus, from a conceptual perspective, Koob emphasized that these stress-driven negative emotional states create an additional source of motivation for drug seeking involving negative reinforcement. Termed "the dark side of addiction," this source of motivation is becoming increasingly recognized as contributing to the deaths of despair involving opioids and alcohol.

Drug cue–induced neuroplasticity

Insights into the physiological processes behind the overwhelming drive in individuals with addiction to seek out a drug and forgo other competing choices were discussed by Peter W. Kalivas (Medical University of South Carolina). When an individual with addiction encounters an external cue or stimulus associated with a drug, such as a call from a friend to meet them at a bar or, in the case of a laboratory animal, a light associated with a drug-delivering lever, cells in the nucleus accumbens are activated, resulting in a cue-specific engram that results in drug-seeking behavior. In individuals without addiction, competing thoughts or cues can alter that response. However, drug cues leave behind long-term potentiation of activity of the nucleus accumbens that blunts the effects of competing stimuli.[70]

Michael Scofield (Medical University of South Carolina) described the mechanism behind this overpotentiation. Normally, when a cue comes to the prefrontal cortex, glutamate is released into the nucleus accumbens, activating a small percentage of neurons, resulting in a stimulus-specific memory trace or engram. Excess glutamate is removed from the synaptic cleft by transporters including GLT-1, a glutamate transporter found on astroglial cells. However, on the basis of animal models of drug addiction, the hypothesis is that GLT-1 is downregulated, and there are fewer astroglial cells in the synaptic cleft.[71] Thus, drug cues cause accumulation of glutamate in the synaptic cleft; subsequent activation of mGluR5, a receptor found on interneurons that express neuronal nitric oxide synthase; release of nitric oxide into the extracellular space; nitrosylation; and activation of matrix metalloproteases (MMPs), especially MMP9, that cause local degradation of the extracellular matrix. This cascade of events provides transient plasticity that contributes to drug-seeking behavior. In withdrawn animals, drug cues result in significant increases in MMP9. Inhibiting MMP9 inhibits drug-seeking behavior in response to drug-associated cues.[72] MMP activity also creates an RGD-binding ligand that activates β3 integrins on spiny neurons, resulting in an increase in spine head diameter and expression of AMPA receptors.[73]

Downregulation of GLT-1 can be found with administration of several classes of addictive substances to animals, including cocaine,[74] nicotine,[70] heroin,[75] and alcohol.[76] An accumulation of glutamate has been observed in animal models of cocaine,[77] nicotine,[70] alcohol,[78] and methamphetamine addictions.[79] Drugs that enhance GLT-1 function, including *N*-acetylcysteine (NAC), ceftriaxone, and propentofylline, have shown positive results in animal models of addiction to cocaine,[80–83] nicotine,[84,85] and alcohol[86] use disorders. NAC has also shown improved behavior in human disorders characterized by intrusive thoughts, such as pathological gambling,[87] trichotillomania,[88] obsessive compulsive disorder (OCD),[89] and depression,[90,91] though there are failures to show improvements in pediatric trichotillomania[92] or methamphetamine addiction.[93] In a recent double-blind, placebo-controlled trial of NAC in veterans with posttraumatic stress disorder (PTSD) and substance

abuse, NAC reduced cravings by week 8. This effect persisted for 4 weeks after stopping NAC. Subjects also reported improvements in CAPS scores of PTSD symptoms and CAPS intrusive thoughts score.[94]

The role of serotonin in anxiety and addiction

Increasing synaptic serotonin levels through the use of selective serotonin reuptake inhibitors (SSRIs), such as Prozac® (fluoxetine) and Zoloft® (sertraline), is a common strategy to relieve anxiety and depression, but the role of serotonin in the brain is complicated.[95,96] First, one of the primary sources of serotonin, the dorsal raphe, projects to areas of the brain involved in impulsivity, reward, stress, anxiety, and feeding. Second, there are many types of serotonin receptors, which can have different effects on behavior. Thomas L. Kash (University of North Carolina School of Medicine) discussed the role of serotonin in increasing anxiety.

Several lines of evidence suggest that increased activation of at least some serotoninergic systems can be highly aversive. SSRI treatment can lead to anxiety, panic, and suicidal ideation in some patients.[97–99] Serotonin has also been shown to play a role in alcohol-induced anxiety. In individuals with AUD, the serotonin agonist metachlorophenylpiperazine has been shown to induce cravings.[100,101] SSRI treatment has been shown to increase anxiety and alcohol consumption in some individuals with AUD.[102,103]

Work from Kash's laboratory has helped to elucidate the neural networks underlying the role of serotonin in alcohol-induced anxiety. In a mouse model of alcohol dependence in which mice were exposed to alcohol vapor and evaluated 24 h after withdrawal, mice displayed increased anxiety-related behaviors.[104] This effect is dependent on serotonin, since injecting the mice with a serotonin receptor antagonist reduced these behaviors.[105] At a neural level, alcohol induced hyperexcitability in both the dorsal raphe, a key source of the serotonin projections to the forebrain, and the bed nucleus of the stria terminalis (BNST), part of the extended amygdala located between the nucleus accumbens and central amygdala and well known for its role in aversive behaviors.

Optogenetic stimulation of serotonin from the dorsal raphe to the BNST also increased anxiety-like behaviors and fear learning in mice. Animals were placed into a chamber where they received a small shock in response to a tone. Stimulating serotonin release optogenetically during the tone resulted in increased freezing behavior, suggesting that serotonin can increase fear recall (data unpublished). A similar study from researchers at Columbia University showed that increasing serotonin levels by injecting fluoxetine into the BNST also enhances fear learning.[106] There is a population of serotonin-responsive neurons in the BNST, which express both CRF and the serotonin 5HT2C receptor. Upon activation, these neurons inhibit neurons that project into the VTA and lateral hypothalamus, thus inhibiting reward-promoting outputs and driving aversive states. Silencing CRF neurons in the BNST blocks the effects of fluoxetine in enhancing fear memory and anxiety.[107]

Transcriptional and epigenetic markers of addiction and implications for treatment

Eric Nestler (Icahn School of Medicine, Mount Sinai) is identifying genes that display changed transcriptional regulation in settings combining social isolation with drug exposure and genes involved in neuronal structure following chronic drug exposure. To measure histone modifications and other chromatin changes, he used RNA sequencing (RNA-seq) to identify changes in RNA levels and chromatin immunoprecipitation (CHiP) followed by deep sequencing (ChiP-seq).

Prior studies have examined the effects of social isolation exposure to addictive drugs administered immediately following the isolation.[108–110] However, discrete periods of stress can lead to long-lasting changes in behavior that can persist even after the stress has been removed. Mice subjected to early-life social isolation followed by weeks of normal, group housing showed altered, sex-dependent cocaine-conditioned place preference compared with controls. Among males, socially isolated mice showed higher place preference than controls, whereas socially isolated female mice showed lower place preference. Since female control mice have higher place preference than male controls, the effects of social isolation appear to equalize behavioral differences between males and females. RNA-seq analysis revealed both sex-specific and housing-dependent differences in gene expression,

particularly in the prefrontal cortex and medial amygdala, but not in the nucleus accumbens or VTA.

Addictive drugs cause structural changes in the neurons of the nucleus accumbens. Chronic cocaine exposure results in longer, thinner, and less functional dendritic spines. During withdrawal, there are increases in more mature, large head spines.[111] In work seeking to identify connections between gene transcription and regulation of the actin cytoskeleton, which mediate spine growth, PDZ-RhoGEF, a RhoA-activating protein, was induced in the nuclei of nucleus accumbens neurons. RhoA increases actin polymerization, decreases the pool of G-actin, and activates serum response factor, a transcription factor that induces transcription of Rap1b in nucleus accumbens neurons. Rap1b is both necessary and sufficient for cocaine-induced formation of thin spines. In behavioral studies, Rap1b knockout blocked the ability of cocaine to produce a strong place preference. Rap1b overexpression increased behavioral responses to cocaine. While increased PDZ-RhoGEF and subsequently increased Rap1b activity is observed 24 h after cocaine withdrawal, this pattern is reversed 3 weeks after withdrawal. These differences help to explain the bidirectional effects of cocaine withdrawal on spiny neurons, though the factors that promote this switch remain unknown.[112]

Yasmin Hurd (Icahn School of Medicine at Mount Sinai Hospital) presented additional data on transcriptional and epigenetic profiles associated with drug use, largely in postmortem human brain specimens. There were significant gene expression differences between heroin users and controls, particularly among genes associated with the glutamatergic system.[113] In addition, there were significant epigenetic differences between the two groups with correlations between epigenetic markers and changes in glutamatergic gene expression. In general, genes associated with the glutamatergic system were hyperacetylated in ways that were likely to indicate greater accessibility and higher transcriptional rates. Expression of the histone acetyltransferase NCOA1 correlated with increased mGluA1 expression among heroin users, but not controls. Epigenetic changes also correlated with years of use, with higher acetylation levels in individuals who had longer histories of heroin use.

These epigenetic changes could be reproduced in a rat model of heroin dependence, suggesting a relationship between heroin use and increased acetylation. Since acetylated histones can be recognized by bromodomain proteins that recruit protein complexes involved in gene expression and since reversible epigenetic marks present opportunities as drug targets, it is notable that bromodomain inhibitors are under investigation in cancer clinical trials.[114] Of functional relevance, the bromodomain inhibitor JQ1 reduced heroin self-administration and drug-seeking behavior in rats.[113]

Personalized treatment for nicotine addiction

Rachel Tyndale (Centre for Addiction and Mental Health, University of Toronto) described how common pharmacogenomic variation in drug-metabolizing genes affects individuals' abilities to quit smoking and/or respond to drug treatments for nicotine dependence. Her hypothesis is that nicotine metabolism plays an important role in smoking behavior. There is a high degree of variability in nicotine metabolism and clearance between individuals, owing primarily to differences in the gene *CYP2A6*,[115–117] which encodes a liver enzyme that metabolizes and inactivates nicotine.[118] Smokers with rapid nicotine metabolism must smoke more to maintain nicotine levels similar to those of slow metabolizers. In one study, smokers with two functioning copies of *CYP2A6* smoked almost 10 more cigarettes per day than those with two defective copies.[119] Slow metabolizers also smoke less intensely, taking slower, more shallow puffs[120] and are more likely to successfully quit smoking, even after controlling for smoking quantity.[121,122] A person's nicotine metabolism rate can also affect how well they respond to smoking cessation therapies. In a trial of bupropion, a dopamine and norepinephrine transport inhibitor, versus placebo, bupropion improved quit rates among fast nicotine metabolizers, but not among slow metabolizers.[123] Bupropion is metabolized by the liver enzyme CYP2B6. Animal and clinical studies suggest that hydroxybupropion, a metabolite of bupropion, may also be an active agent for smoking cessation success, as higher levels of hydroxybupropion correlate with higher quit rates.[124] Genetic variants in *CYP2B6* that affect the rate of bupropion metabolism have been identified. Therapeutic drug monitoring of hydroxybupropion or *CYP2B6*

genotyping could be useful to guide individual dose adjustments in patients taking bupropion.

While bupropion is more effective among fast nicotine metabolizers, randomized trials have shown that the nicotine patch is more effective among the slow nicotine metabolizers than fast metabolizers. More rapid nicotine clearance would be expected to decrease nicotine levels in people using nicotine replacement therapies, such as the patch, thereby limiting their effectiveness in smoking cessation.[125,126] In a randomized study comparing efficacies of the nicotine patch, varenicline, a nicotinic receptor partial agonist, or placebo in smoking cessation, varenicline treatment was more effective than the patch among normal nicotine metabolizers, while the two treatments were equally effective in slow metabolizers.[127] On the basis of these results, it may be possible to personalize smoking cessation treatment based on the *CYP2A6* genotype. The nicotine patch may be appropriate for slow metabolizers as it is effective, inexpensive, and has a low side effect profile. Varenicline may be more appropriate for normal metabolizers, as it was more effective than the patch in this group.

One remaining question is how nicotine metabolism affects smoking rates weeks to months after nicotine has cleared the body. Some clues have emerged from brain imaging studies. Cue-evoked images had larger effects on brain activity in fast metabolizers compared with slow metabolizers among active smokers, suggesting that differences in nicotine metabolism may have long-lasting effects on the brain.[128]

Effects of addictive drugs on the developing brain: adolescents and young adults

Diana Fishbein (Pennsylvania State University) described adolescence as a period of significant, rapid brain development during which the adolescent brain provides increased addiction-related risks. The last region of the brain to develop is the frontal cortex, responsible for executive functions that include impulse control, risk determination, evaluation of consequences, and decision making. During adolescence and into the early 20s, there are significant changes in both gray and white matter in the frontal cortex, including continued myelination, gray matter thinning, and pruning of excess connections established earlier in development.[4,129] The prefrontal cortex also serves to modulate the activities of noncortical systems, such as regulating emotional circuit activity in the limbic system. Imaging studies have revealed that the dopaminergic connectivity to the frontal cortex is weaker in children than in adults. As the prefrontal cortex matures during adolescence, there is a linear increase in inhibitory control. However, there is also an increased activity in the nucleus accumbens, which increases reward sensitivity.[130] These differences are likely to contribute to the greater risk-taking behavior, novelty seeking, and impulsivity that can be observed in adolescence.

Because their brains are still developing, adolescents are thus more likely to both engage in risk-taking behaviors, including drug self-administration, and display enhanced vulnerability to the effects of drugs and alcohol. Large epidemiological studies show that adolescents and young adults are more likely to start using drugs than older adults. Adolescents are also more likely to transition from experimenting with drugs to develop substance use disorders.[131] In the National Longitudinal Alcohol Epidemiologic Survey, 45% of people who began drinking before the age of 14 grew up to ultimately have an AUD, compared with 10% of people who began drinking after the age of 21.[132] The more recent National Epidemiologic Survey on Alcohol and Related Conditions showed that those who started drinking at an early age were more likely to experience alcohol dependence within 10 years of beginning drinking.[133]

The effects of alcohol on brain development

Alcohol use among adolescents has been associated with differences in and/or changes in brain structure and function.[134] Fifteen- and 16-year-olds enrolled in an alcohol treatment program showed about a 10% deficiency in memory compared with non-drinking, matched controls as well as differences in cerebellar, hippocampal, and prefrontal cortex volume and white matter quality.[135]

Susan Tapert (University of California, San Diego) described work on the Youth at Risk Study to elucidate how adolescents who use drugs and alcohol differ from those who do not. In this longitudinal study of 300 middle schoolers who had

not started drinking at the time of study entry, 40% remained nondrinkers, 30% drank modestly or moderately, and 30% had become heavy drinkers when studied 4 years later. Adolescents who began drinking during the follow-up period performed worse on several measures of neurocognition compared with those who did not start drinking. Girls who started drinking showed greater impairment in memory tests; boys did worse on tests of visual attention.[136] Extreme binge drinking was associated with poorer performance on several measures of verbal learning and memory compared with subjects who engaged in little or no binge drinking.[137]

Several differences in brain structure and activity were also observed. Adolescents who initiate heavy drinking showed accelerated reduction of gray matter volumes, particularly in temporal and lateral frontal areas, and attenuated growth in white matter. Structural MRI data also reveal greater reductions in brain volume in the left temporal lobe, caudate, thalamus, and brain stem.[138]

Functional MRI studies reveal that teens who become heavy drinkers display lower levels of brain activity during visual working memory tasks even before they start drinking, without any deficit in performance.[139] This suggests that they are not as cognitively involved in the nonrewarding task as those who do not become drinkers. After they become drinkers, however, their brain activity increases during these tasks, likely indicating that their brains are working harder to do the same task than their nondrinking counterparts.

Lindsey Squeglia (Medical University of South Carolina) described baseline characteristics that predicted future heavy drinking. Demographic variables, including male gender and higher socioeconomic status, were associated with higher risk of becoming a heavy drinker, as were behaviors that included conduct disorders. In addition, several neuropsychological and imaging characteristics differed at baseline in future heavy drinkers. Subjects who began drinking had pre-existing smaller volumes in the inferior frontal cortex, cingulate, and cerebellum. They also had thinner cortex in several brain regions and showed less brain activity during a working memory task. A predictive model consisting of 38 variables—demographic, behavioral, neuropsychiatric, and imaging—could predict future heavy drinking with 74% accuracy. Understanding the risk factors for heavy drinking among teens can help pediatricians and counselors.[140]

Functional MRI studies have also shed light on the role that the media and advertising may play in substance use initiation. A group of 15- to 18-year-olds composed of heavy binge drinkers and moderate, nonbinge drinkers was shown a series of advertisements for alcoholic and nonalcoholic products. Heavy drinkers showed greater brain activation when looking at the alcohol ads compared with nonalcohol ads. Conversely, the nonbinge drinkers showed no difference in brain activation for the two types of ads.[141] Abstaining from alcohol for 5 weeks was able to attenuate this response, suggesting that the response may be reversible.[142]

Two large-scale, longitudinal studies are underway to better understand the effects of alcohol and/or other abused substances on neurodevelopment in adolescents. The National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) has recruited over 800 adolescents from ages 12 to 21, many of them at risk of substance use, among five sites in the United States by 2016. Subjects are being followed up annually with neuroimaging and neurophysiological testing.[143] A second, larger study, the Adolescent Brain Cognitive Development (ABCD), is following over 11,000 9- to 10-year-olds for 10 years in 19 sites around the United States. Enrollment of the study began in 2016, and as of early 2018, over 11,000 participants have been enrolled, with baseline data available to researchers for 4500 participants.[144,145]

In utero exposure to cannabis

Yasmin Hurd (Icahn School of Medicine at Mount Sinai Hospital) noted that cannabis use is relatively common among pregnant women. In a retrospective study of newborn drug testing in the United States, nearly 20% of fetuses tested positive for THC.[146] The long-term implications of this exposure are unclear. In postmortem brain samples of human fetuses, Hurd showed that exposure to cannabis *in utero* was associated with lower levels of the dopamine D2 receptor in the amygdala and nucleus accumbens. Lower expression correlated with more maternal smoking.[147] Similar expression patterns are observed in animal models, which last into adulthood.[148]

Perhaps most strikingly, cannabis use may even affect future generations even without direct

exposure. In rats, exposure to cannabis during adolescence has epigenetic and behavioral effects in unexposed offspring and later generations. Offspring of parents exposed to THC were more likely to self-administer heroin and had changes in DNA methylation in genes associated with synaptic plasticity, psychiatric disorders, and neurodevelopment.[149]

Effects of marijuana on the adolescent brain

In 2015, daily marijuana use (6%) surpassed daily cigarette use (5.5%) among high school seniors. At the same time, the perception of risk and harm associated with marijuana among high schoolers fell to an all-time low (29%).[150] Understanding the effects of marijuana on this vulnerable population will continue to be important as legalization efforts continue across the country.

While several studies have compared the effects of marijuana use on brain structure and function in smokers versus nonsmokers, few have looked at whether the age at which a person starts smoking cannabis comes into play. Staci Gruber (McLean Hospital, Harvard Medical School) investigated whether the age of onset of marijuana use affects neurocognitive performance, brain function, and brain structure. In fMRI studies, late-onset smokers had activation patterns that were more similar to control, nonsmokers than to early-onset smokers. Adolescents who began smoking earlier (before 16 years of age) smoked nearly twice as often and more than 2.5 times as much as those who began smoking later. Earlier age of onset was also associated with poorer performance on measures of executive function than late age of onset.[5,151]

Marijuana users also exhibit differences from controls in brain volume, mass, and shape. There are regional differences in cerebral cortical thickness compared with nonusers and differences in density and in gyrification, a measure of the folding of the cortex in the gray matter that has been related to poor performance on attentional tasks. Among early-onset smokers, significant reductions in white matter integrity have been observed by diffusion tensor imaging compared with images from late-onset smokers and nonsmokers. This difference in white matter integrity was associated with higher self-reported impulsivity among early-onset smokers, but not among late-onset smokers or nonsmoking controls,[152] although the causality of this relationship is not fully understood.

Susan Weiss also noted both the importance of determining the effects of marijuana on the developing brain, given its increasing prevalence and availability to adolescents, and the lack of consistency in the field. For example, a large longitudinal study in New Zealand reported that persistent cannabis use was associated with a decline in IQ;[153] however, twin studies failed to observe this connection.[154,155] Studies of the effects of marijuana on brain structure are also mixed.[156] Gaps in knowledge include whether the effects of marijuana are reversible with abstinence; how varying doses, strains, and potency of cannabis affect outcomes; whether there are gender-specific effects; and how the age of onset influences cannabis effects.

Electronic cigarettes and adolescents

Thomas Eissenberg (Virginia Commonwealth University) noted that according to National Youth Tobacco Surveys, while cigarette use has declined among high schoolers,[157] e-cigarette use has been steadily increasing.[158] E-cigarettes are now the most popular tobacco product among U.S. adolescents with 16% of high schoolers reporting recent e-cigarette use.[158] E-cigarettes represent a constantly evolving class of devices for which there are currently few standards and only emerging regulation; several FDA-announced regulations are being challenged by e-cigarette companies.[159–161]

Eissenberg expressed concern that e-cigarettes are being marketed to young consumers with flavors like blue cotton candy, applejack, and hard candy. Advertisements geared toward young adults focus on low nicotine products, which may function as gateway products. These devices deliver nicotine poorly, giving new users opportunities and chances to try products and experiencing some positive reinforcement without the acute nicotine toxicity that they might experience with more potent products.[162–164]

E-cigarettes are often marketed as a safer alternative to cigarettes, with a focus on reduced risk of lung cancer. However, Olusegun Owotomo (University of Texas at Austin) stressed that nicotine addiction is an important health risk of e-cigarettes. He showed that among a nationally representative sample of 8th and 10th graders from the Monitoring the Future Study, adolescent e-cigarette users endorsed a number of attitudes, perceptions, and characteristics

that are risk factors for cigarette smoking compared with nonusers.[165]

The variability between products in relation to construction, power, and components in the e-liquid makes it difficult to study e-cigarettes as a class. In addition, e-cigarette liquid usually contains flavorants intended for consumption that have not been tested for inhalation safety. Finally, the presence of other potentially toxic compounds, such as aerosolized propylene glycol or formaldehyde, depends on the type of device and liquid used. Unsurprisingly, there are few data on the long-term health effects of e-cigarettes. Indeed, such data will likely be difficult to gather given the variability not only between devices but also between users.

Commercial e-cigarette products vary widely with respect to how much nicotine they deliver, with some devices delivering more nicotine than a conventional combustible cigarette. Factors such as construction, battery power, the liquid used, and user behavior can significantly affect the amount of nicotine that is delivered to the smoker.[166–169] Increasing the battery wattage by a factor of two can quadruple the amount of nicotine delivered.[170] Level of experience can also affect nicotine delivery. Cigarette smokers trying e-cigarettes for the first time were not as efficient as experienced e-cigarette smokers. Experienced smokers take drags that are twice as long as those of new smokers, keeping the heating element activated for longer time periods and delivering more nicotine.[171]

Using neuroscience to tailor drug prevention programs

Diana Fishbein (Pennsylvania State University) described some of the efforts to use neuroscience research in drug prevention programs. Exciting developments in neuroscience have the potential to inform the development of preventive antismoking interventions in a more targeted, precision-based manner. While many evidence-based prevention interventions have been shown to be effective, the effects are often modest.

Research on neural networks, genetics, and epigenetics should help to lead to tailored and targeted interventions. One area that has shown to be effective is targeting of stress regulatory systems. Chronic stress can prime the brain for novelty seeking and drug use.[172] Interventions that target stress physiology and neural markers have shown some efficacy in behavioral change. Mindfulness programs have the potential to affect brain function and structure across age groups.[173–176] The PATHS curriculum increases social competence and decreases behavioral problems.[177] The Early Risers program can promote executive function and reduce conduct problems among homeless youth.[178] The Head Start REDI program promotes gains in executive function that partially mediate school readiness among kindergarten children.[179] Interventions have even shown to improve physiological markers of stress. A family-based intervention conducted in young foster children normalized cortisol levels and improved hypothalamic-pituitary-adrenal axis functioning.[180]

Newer approaches to studying addiction: optogenetics

Optogenetics takes advantage of light-sensitive ion channels to perturb neural circuits by either depolarizing or hyperpolarizing neurons. The most common ion channel used is the H134 channelrhodopsin, which opens when illuminated with blue light and depolarizes neurons when optically stimulated. Inhibitory pumps and G-protein-coupled receptor/rhodopsin chimeras can also be used. While the animal is traditionally tethered to a fiber optic cable inserted into the expressing brain region, this restraint can limit the types of behavior and analyses that can be conducted.[181] Similar limitations can come from traditional drug self-administration apparatus.

Michael Bruchas (Washington University School of Medicine) and John Rogers (Northwestern University) have developed wireless, implantable LED devices that obviate the need for tethering animals and provide other advantages.[182] LEDs are printed onto a neural probe along with temperature sensors, photo detectors, and electrodes so that the final product measures only approximately 25 $\times$ 25 μm. The LEDs are controlled wirelessly via radio frequency. This technique allows much more freedom for measuring animal behavior in response to neural circuit perturbations. Animals can be studied in their home cages, without being handled by humans, thus expanding the ranges of behavioral tests to, for example, light/dark box assays that assess anxiety-like behaviors.

The LED device does not generate substantial heat and does not change the temperature of the brain. LED probes can activate channel rhodopsins and chimeric opto-XR receptors to activate G-protein-coupled receptor signaling pathways. These probes can also administer drugs to specific areas of the brain. Previous methods of administering drugs to the brain involved hooking an animal up to a pump via a cannula and infusing a drug into different areas of the brain. LED probes with fluidic channels can infuse agents adjacent to the LED, thus adjacent to the area of the brain that will be photo-stimulated. The same wireless platform can power both the LED and the drug infusion, allowing researchers to combine optogenetics and pharmacology in awake animals with minimal handling.[183]

Newer approaches to studying addiction: deep brain stimulation

While optogenetics has proven to be a useful tool in the laboratory, it is currently less feasible as a treatment in humans. Deep brain stimulation (DBS) involves sending electrical impulses to specific areas of the brain via implanted electrodes. DBS is currently used in the treatment of a number of neurological conditions, especially Parkinson's disease, but also epilepsy and OCD.

Meaghan Creed (University of Geneva) uses DBS to depotentiate synapses in the nucleus accumbens. DBS has been shown to be effective in abolishing some of the neurological and behavioral effects of cocaine in mice. The background for this work comes from findings that addictive drugs alter both the quality and the quantity of synaptic transmission in the D1 receptor expressing spiny neurons of the nucleus accumbens. These changes persist long after the drug is out of the system. One of the consequences of the strong dopaminergic response stimulated by cocaine and other drugs of abuse is a switch in the D1 medium spiny neurons in the nucleus accumbens from $GluA2^{+}$ AMPA receptors to $GluA2^{-}$ AMPA receptors. This enhances the strength of the excitatory transmission onto D1 MSNs and over-potentiates the synapse.[184–186] Depressing or desensitizing the synapse may be able to reverse the effects of addictive drugs. Optogenetic stimulation has been shown to reverse cocaine-induced synaptic plasticity in mice both with reference to synaptic strength and the composition of AMPA receptors. In addition, optogenetic stimulation abolished cocaine-induced hyperactivity.[187]

Creed's laboratory, using DBS at a frequency similar to that used in Parkinson's disease, reduced synaptic strength and hyperactivity in mice in response to cocaine; however, the effects were transient. The stimulation was ineffective if the cocaine was administered as little as 4 h after DBS. Lower frequencies, similar to those used in optogenetics experiments, showed no effects on synaptic strength or behavior. DBS thus may be nonspecifically stimulating several inputs in the brain. Stimulating dopamine signaling may cancel the intended dopamine-lowering effect. Adding a dopamine antagonist to low-frequency DBS, optogenetically inspired DBS (oiDBS), significantly suppressed cocaine-induced hyperactivity and reversed cocaine-induced synaptic plasticity. Importantly, the effects of a single 10-min oiDBS session persisted for at least 1 week. Subsequent work revealed that the effects of oiDBS are dependent on mGluR, since pretreatment with an mGluR blocker abolished the oiDBS effects.[187]

Newer approaches to treating addiction: drug vaccines

Ron Crystal (Weill Cornell Medical College) described work in developing vaccines against addictive drugs that would prevent them from entering and thus affecting the brain. Addictive drugs are small molecules that are not highly immunogenic, however. Though the immune system does not readily produce good antibodies directed against addictive drugs, this hurdle is being addressed via two approaches.

Active vaccination strategies conjugate the addictive drug to adenovirus capsid proteins, which are highly immunogenic. Crystal has developed a cocaine vaccine by conjugating the cocaine analog GNE to adenovirus that has been denatured so that it cannot replicate.[188] The vaccine, dAd5GNE, can engender high anticocaine antibody titers in both rodent and nonhuman primate models.[189] The vaccine prevents cocaine distribution in the brain in rodent models, even with frequent administration and at very high doses.[190] It also reduces cocaine self-administration in nonhuman primates.[191]

A phase 1 clinical trial is underway in human cocaine users. Participants receive 6 monthly

injections of either conjugate vaccine or placebo. The study will investigate the ability of three different vaccine doses to produce anticocaine antibodies and will assess safety.[192]

In passive immunization approaches, expression of a gene that encodes an anticocaine antibody is delivered to the liver using an adeno-associated virus vector. Transfection with the AAvrh.10 vector containing this anticocaine antibody gene can produce high, persistent anticocaine antibody titers following a single administration to animals. In mice, AAvrh.10 reduced cocaine levels in the brain and reduced cocaine-induced hyperactivity for periods of months after the transfection.[193]

Pharmacologic agents for opioid addiction

Over the past decade, the number of heroin users has increased significantly, while the number of deaths due to heroin and prescription opioids has increased fivefold.[194–196] David Gastfriend (American Society of Addiction Medicine) noted that pharmacotherapy can be an important component of a successful treatment program, that multiple agents approved for opioid dependence provide different advantages to suit patients' needs, but that there is inadequate use of pharmacotherapy to treat opioid dependence. Of the 2.5 million Americans who abused or were dependent on opioids in 2012, fewer than 1 million received medication-assisted therapy.[197]

The three approved agents for opioid dependence—methadone, buprenorphine, and naltrexone—each has different characteristics in practice. Methadone is the most tightly controlled and least accessible of the three, dispensed by the 1300 certified methadone clinics in the United States. In randomized controlled trials, methadone treatment has been shown to stabilize people in recovery and to reduce harms including HIV and HCV transmission.[198] After terminating methadone treatment, 82% of patients return to heroin use within a year.[199] Because of this, methadone is best used as a long-term treatment. Because of its tightly controlled access, patients with more chaotic lifestyles who need close, daily supervision and who are prepared for long-term treatment, including those with psychiatric illness or a high tolerance for opioids, may be suitable candidates for methadone treatment.

For patients with more structured lives who can maintain treatment plans without daily monitoring, buprenorphine or buprenorphine/naloxone combinations may be suitable options. While a physician must be licensed to prescribe buprenorphine, any physician can apply to be a buprenorphine prescriber. Retention can be an issue. In one study of HIV-infected opioid-dependent patients, 1-year retention in buprenorphine/naloxone treatment was only 49%.[200] Several studies have shown mean retention rates of 2–3 months.[201,202] Up to 92% of patients relapse within 8 weeks of tapering treatment.[203] A meta-analysis of 31 trials showed that methadone maintenance therapy had higher retention rates than low-dose or flexible-dose buprenorphine therapy. However, fixed medium or high buprenorphine doses, though less common in office-based clinical practice, were equivalent to methadone in rates of retention in treatment and suppression of illicit drug use.[204] Buprenorphine may disrupt cognitive function less often than methadone, especially early in treatment. During the first months of treatment, patients on methadone show greater delays in reaction time than controls.[205] After maintenance is established, this discrepancy is diminished but is still present for several measures of cognitive function.[206–209]

The newest agent available for opioid dependence, the antagonist naltrexone, was recently approved as an extended-release formulation administered as a monthly injection. Naltrexone XR is a significant improvement over short-acting oral naltrexone, which failed to improve retention or abstinence[210] rates more than placebo and was associated with three- to sevenfold higher death rates than methadone.[211] In brain imaging studies, naltrexone XR modulates the brain's response to drug cues in abstinent heroin-dependent patients.[212] There were both activity decreases in limbic regions and activity increases in areas involved in self-reflection and self-regulation, including the medial frontal gyrus. In a double-blind, placebo-controlled trial, naltrexone XR treatment provided significantly higher rates of abstinence, decreases in cravings, and higher retention rates.[213] Naltrexone XR has also been shown to be more cost effective than methadone or buprenorphine. Although naltrexone XR is more expensive, overall healthcare costs, including inpatient, outpatient, and addiction

care, are significantly lower in patients treated with naltrexone XR (US$8,582 in the first 6 months) compared with methadone (US$16,752).[202]

Because naltrexone requires that patients be opioid free before beginning treatment, it is most appropriate for motivated patients who are dedicated to undergo detox and who have structure and support systems in place. A theoretical concern with naltrexone treatment is that the abstinence associated with naltrexone ablates opioid tolerance in ways that may predispose to increased overdose risks if treated patients relapse to opioid use. In clinical trials, however, no overdose-related deaths have been observed up to 18 months.[214,215]

Monitoring treatment efficacy for opioid use disorder

Silvia Lopez-Guzman (New York University) described research detailing how impulsivity can predict relapse in patients undergoing treatment for opioid dependence. In a meta-analysis of 46 studies, temporal discounting (a measure of the value of a reward that is available now versus one available at different times in the future) was consistently higher in substance users for several drugs of abuse.[216] There is also evidence that treatment for opioid dependence may reduce impulsivity.[217] In a longitudinal study of patients starting treatment for opioid dependence and matched controls, Lopez-Guzman measured each subject's impulsivity at several time points using a model of temporal discounting in which the person is asked to decide between receiving a small sum of money now or a larger sum at some point in the future. For individuals with high impulsivity, rewards lose their value more quickly over time, that is, the longer they must wait to receive a reward, the less valuable it is to them. While baseline impulsivity levels did not predict relapse rates, patients who experienced increased impulsivity during the trial often relapsed. Impulsivity-rate increase correlated with an increase in relapse and may be a marker for patients at risk of relapse. Conversely, a decrease in impulsivity over time was associated with reduced relapse and may be a signal of recovery or resiliency.

Since impulsivity can be monitored by smartphone or tablet apps, testing could be useful to identify patients who are responding well to opioid-dependence treatment versus those at elevated risk of relapse.

Public policy considerations for addiction: legalized marijuana

Susan Weiss (National Institute on Drug Abuse) summarized thoughts that much of the current research into effects of policy is inconclusive as it fails to take into account heterogeneity between states with regard to how new policy laws are implemented. In addition, the measures being collected, namely prevalence of drug use, do not always correspond to measures of harm, such as hospitalizations. State legislatures legalizing marijuana are not using evidence-based policy research from tobacco and alcohol control. The legalization process, such as regulations on advertising, pricing, taxes, and potency, can have significant effects on mitigating the harm of legalized marijuana.

Benefits and harms of public health policies

Mark Kleiman (New York University) discussed the societal effects of addiction and public policies aimed to curb addiction. It is clear that addiction and drugs of abuse can have negative consequences on public safety and public health, whether in the form of overdose, sexually transmitted diseases, violence, or drunk driving. What may be less clear, however, is that the public policies put in place to mitigate these harms may have unintended consequences of their own. For example, forbidding the use of illicit drugs often creates illicit markets, which carry risks in terms of drug-related violence, police enforcement, and incarceration. Therefore, when thinking about public policy, Kleiman stressed that it is important to take a holistic view and realize that drug-control policies can increase harm, even as they attempt to decrease use.

Kleiman advised that there are several strategies for curtailing drug use, including levying taxes, marketing and antimarketing campaigns, and sobriety programs. These methods can have a significant effect on overall public health. For example, the Sobriety 24/7 program, which establishes zero tolerance for repeat drug and alcohol-related offenders, was shown to reduce automobile crashes by 12%, domestic violence reports by 8%, and all-cause mortality by 4%.[218]

The ongoing cannabis legalization efforts offer an opportunity for policymakers to learn from lessons in alcohol and tobacco regulation.

Ann. N.Y. Acad. Sci. 1451 (2019) 5–28 © 2019 The Authors. *Annals of the New York Academy of Sciences* published by Wiley Periodicals, Inc. on behalf of The New York Academy of Sciences.

However, Kleiman argues that, owing to the influence of corporate interests, which focus on the small percentage of heavy (and profitable) users, effective measures to moderate cannabis use have not been implemented. Such measures could include limiting sales to government stores, high taxation to prevent price collapse, implementing standardized measures of cannabis intake, regulating the concentrations of THC and cannabidiol, and requiring users to pass a cannabis test and receive a license to become a registered cannabis user, similar to a driver's license.

Improving the state of mental health care

Patrick J. Kennedy (One Mind, former United States representative from Rhode Island) oversaw the passage of the Mental Health Parity and Addiction Equity Act of 2008 (MHPAEA), which requires health insurance companies to cover mental health and substance use disorders in the same manner as physical disorders and diseases. Issues with the current state of mental health and addiction care include a need for more physicians willing to treat addiction, the lack of coordinated and integrated care with a patient's primary provider, and the need to incorporate proven, evidence-based methods into treatment. Public perception needs to change to view addiction as a chronic disease. Like other chronic diseases, there is no cure for addiction. Therefore, retention in addiction treatment programs is critical. In addition, the care model for chronic diseases, in which patients are continually monitored to assess the effectiveness of treatment, and treatment plans are modified in accordance with measurable markers of disease control, should be adopted for addiction treatment.

There are cautions concerning roles for increasingly large marijuana companies in the current cannabis legalization processes. Similar to what has been seen with the tobacco and alcohol industries, the marijuana industry may be targeting young, poor, and minority customers.[219,220] Legalization has not improved the potential negative impact of marijuana on these populations. In Colorado, for example, marijuana-related arrests among Black and Hispanic youth are higher after the legalization of cannabis than they were before.[221] With the state of mental health care in this country, addiction can be devastating. Kennedy warned that "we cannot stand by and watch a new industry like Big Tobacco take over and addict poor people who don't have access to [comprehensive addiction treatment]."

Conclusions

Attendees at the Sixth Annual Aspen Brain Forum "The Addicted Brain and New Treatment Frontiers" were privileged to hear NIH institute directors and political leaders join with clinical, translational, and basic researchers to discuss their varying perspectives on the neurobiology of addiction. Therapeutic opportunities and increased political and regulatory sophistication have been brought to bear on the challenge of diagnosing, preventing, and treating addictions. The magnitude of the human burden of the disease of addiction and timeliness of the challenges that addiction provides were never far from even the more technical scientific discussions. Hope, excitement, and challenges animate this field and are all found in this exceptional conference.

Acknowledgments

This report was written to reflect topics presented at the Sixth Annual Aspen Brain Forum, "The Addicted Brain and New Treatment Frontiers" (https://www.nyas.org/events/2016/the-addicted-brain-and-new-treatment-frontiers-sixth-annual-aspen-brain-forum/?tab=sponsors), which convened at the New York Academy of Sciences May 18–20, 2016. Publication of the report satisfies requirements for disseminating the forum's content. All topics discussed by the authors (J.C., G.R.U., and G.F.K.) reflect their interpretations of the speakers' presentations at the forum in 2016. Open Access of the report was supported by the National Institute on Drug Abuse of the National Institutes of Health, Award Number R13DA041813.

Competing interests

G.F.K. directs the National Institute on Alcoholism and Alcohol Abuse, which funded significant portions of the work reported herein.

Author contributions

The initial draft of this report was developed by J.C. on the basis of audio recordings of the presentations. For readability, presentations are grouped thematically, as opposed to order of presentation. Works cited in the presentation are referenced in this report, where possible; in some cases, statistics have been updated to reflect the most recent data. G.K.

and G.U. corrected and edited subsequent drafts of the report to incorporate their reflections from the presentations.

References

1. Bose, J., S.L. Hedden & R.N. Lipari. 2018. *Key Substance Use and Mental Health Indicators in the United States: Results from the 2017 National Survey on Drug Use and Health.* Rockville, MD: Center for Behavioral Health Statistics and Quality.
2. Bahorik, A.L., D.D. Satre, A.H. Kline-Simon, *et al.* 2017. Alcohol, cannabis, and opioid use disorders, and disease burden in an integrated health care system. *J. Addict. Med.* **11:** 3–9.
3. National Institute on Drug Abuse. Trends & statistics. April 2017.
4. Giedd, J.N. 2015. Adolescent neuroscience of addiction: a new era. *Dev. Cogn. Neurosci.* **16:** 192–193.
5. Sagar, K.A., M.K. Dahlgren, A. Gönenç, *et al.* 2015. The impact of initiation: early onset marijuana smokers demonstrate altered Stroop performance and brain activation. *Dev. Cogn. Neurosci.* **16:** 84–92.
6. Volkow, N.D., G.-J. Wang, J.S. Fowler, *et al.* 2011. Addiction: beyond dopamine reward circuitry. *Proc. Natl. Acad. Sci. USA* **108:** 15037–15042.
7. Volkow, N.D., G. Wang, J.S. Fowler, *et al.* 2001. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J. Neurosci.* **21:** RC121.
8. Grace, A.A. 2000. The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction* **95**(Suppl. 2): S119–S128.
9. Baik, J.-H. 2013. Dopamine signaling in reward-related behaviors. *Front. Neural Circuits* **7:** 152.
10. Volkow, N.D. & M. Morales. 2015. The brain on drugs: from reward to addiction. *Cell* **162:** 712–725.
11. Volkow, N.D., D. Tomasi, G.-J. Wang, *et al.* 2014. Stimulant-induced dopamine increases are markedly blunted in active cocaine abusers. *Mol. Psychiatry* **19:** 1037–1043.
12. Volkow, N.D., G.J. Wang, J.S. Fowler, *et al.* 1997. Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature* **386:** 830–833.
13. Volkow, N.D., J.S. Fowler, G.-J. Wang & R.Z. Goldstein. 2002. Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. *Neurobiol. Learn. Mem.* **78:** 610–624.
14. Thanos, P.K., N.D. Volkow, P. Freimuth, *et al.* 2001. Overexpression of dopamine D2 receptors reduces alcohol self-administration. *J. Neurochem.* **78:** 1094–1103.
15. Thanos, P.K., N.B. Taintor, S.N. Rivera, *et al.* 2004. DRD2 gene transfer into the nucleus accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. *Alcohol. Clin. Exp. Res.* **28:** 720–728.
16. Robertson, C.L., K. Ishibashi, J. Chudzynski, *et al.* 2016. Effect of exercise training on striatal dopamine D2/D3 receptors in methamphetamine users during behavioral treatment. *Neuropsychopharmacology* **41:** 1629–1636.
17. Montague, P.R., S.E. Hyman & J.D. Cohen. 2004. Computational roles for dopamine in behavioural control. *Nature* **431:** 760–767.
18. Lohrenz, T., K. McCabe, C.F. Camerer & P.R. Montague. 2007. Neural signature of fictive learning signals in a sequential investment task. *Proc. Natl. Acad. Sci. USA* **104:** 9493–9498.
19. Chiu, P.H., T.M. Lohrenz & P.R. Montague. 2008. Smokers' brains compute, but ignore, a fictive error signal in a sequential investment task. *Nat. Neurosci.* **11:** 514–520.
20. Kishida, K.T., I. Saez, T. Lohrenz, *et al.* 2016. Subsecond dopamine fluctuations in human striatum encode superposed error signals about actual and counterfactual reward. *Proc. Natl. Acad. Sci. USA* **113:** 200–205.
21. Mackie, K. 2006. Cannabinoid receptors as therapeutic targets. *Annu. Rev. Pharmacol. Toxicol.* **46:** 101–122.
22. GW Pharm Plc. 2016. Sativex (delta-9-tetrahydro cannabinol and cannabidiol).
23. GW Pharm Plc. 2014. GW Pharmaceuticals announces that Sativex receives fast track designation from FDA in cancer pain.
24. Devinsky, O., M.R. Cilio, H. Cross, *et al.* 2014. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* **55:** 791–802.
25. Justinova, Z., L.V. Panlilio, G. Moreno-Sanz, *et al.* 2015. Effects of fatty acid amide hydrolase (FAAH) inhibitors in non-human primate models of nicotine reward and relapse. *Neuropsychopharmacology* **40:** 2185–2197.
26. Lazary, J., N. Eszlari, G. Juhasz & G. Bagdy. 2016. Genetically reduced FAAH activity may be a risk for the development of anxiety and depression in persons with repetitive childhood trauma. *Eur. Neuropsychopharmacol.* **26:** 1020–1028.
27. Piomelli, D., G. Tarzia, A. Duranti, *et al.* 2006. Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS Drug Rev.* **12:** 21–38.
28. Wei, D., D. Dinh, D. Lee, *et al.* 2016. Enhancement of anandamide-mediated endocannabinoid signaling corrects autism-related social impairment. *Cannabis Cannabinoid Res.* **1:** 81–89.
29. Di Forti, M., C. Iyegbe, H. Sallis, *et al.* 2012. Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biol. Psychiatry* **72:** 811–816.
30. Di Forti, M., A. Marconi, E. Carra, *et al.* 2015. Proportion of patients in south London with first-episode psychosis attributable to use of high potency cannabis: a case–control study. *Lancet Psychiatry* **2:** 233–238.
31. Arseneault, L., M. Cannon, R. Poulton, *et al.* 2002. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ* **325:** 1212–1213.
32. Whitelaw, R.B., A. Markou, T.W. Robbins & B.J. Everitt. 1996. Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement. *Psychopharmacology (Berl)* **127:** 213–224.

33. Ito, R., J.W. Dalley, T.W. Robbins & B.J. Everitt. 2002. Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. *J. Neurosci.* **22:** 6247–6253.
34. Robbins, T.W. & B.J. Everitt. 1999. Drug addiction: bad habits add up. *Nature* **398:** 567–570.
35. Everitt, B.J. & T.W. Robbins. 2005. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat. Neurosci.* **8:** 1481–1489.
36. Everitt, B.J., A. Dickinson & T.W. Robbins. 2001. The neuropsychological basis of addictive behaviour. *Brain Res. Brain Res. Rev.* **36:** 129–138.
37. Ito, R., J.W. Dalley, S.R. Howes, *et al.* 2000. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J. Neurosci.* **20:** 7489–7495.
38. Vanderschuren, L.J.M.J., P. Di Ciano & B.J. Everitt. 2005. Involvement of the dorsal striatum in cue-controlled cocaine seeking. *J. Neurosci.* **25:** 8665–8670.
39. Adams, C.D. & A. Dickinson. 1981. Instrumental responding following reinforcer devaluation. *Q. J. Exp. Psychol.* **33:** 109–122.
40. Zapata, A., V.L. Minney & T.S. Shippenberg. 2010. Shift from goal-directed to habitual cocaine seeking after prolonged experience in rats. *J. Neurosci.* **30:** 15457–15463.
41. Olmstead, M.C., M.V. Lafond, B.J. Everitt & A. Dickinson. 2001. Cocaine seeking by rats is a goal-directed action. *Behav. Neurosci.* **115:** 394–402.
42. Olmstead, M.C., J.A. Parkinson, F.J. Miles, *et al.* 2000. Cocaine-seeking by rats: regulation, reinforcement and activation. *Psychopharmacology (Berl)* **152:** 123–131.
43. Corbit, L.H., H. Nie & P.H. Janak. 2012. Habitual alcohol seeking: time course and the contribution of subregions of the dorsal striatum. *Biol. Psychiatry* **72:** 389–395.
44. Corbit, L.H., H. Nie & P.H. Janak. 2014. Habitual responding for alcohol depends upon both AMPA and D2 receptor signaling in the dorsolateral striatum. *Front. Behav. Neurosci.* **8:** 301.
45. Clemens, K.J., M.R. Castino, J.L. Cornish, *et al.* 2014. Behavioral and neural substrates of habit formation in rats intravenously self-administering nicotine. *Neuropsychopharmacology* **39:** 2584–2593.
46. Belin, D. & B.J. Everitt. 2008. Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron* **57:** 432–441.
47. Murray, J.E., A. Belin-Rauscent, M. Simon, *et al.* 2015. Basolateral and central amygdala differentially recruit and maintain dorsolateral striatum-dependent cocaine-seeking habits. *Nat. Commun.* **6:** 10088.
48. Gremel, C.M. & R.M. Costa. 2013. Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. *Nat. Commun.* **4:** 2264.
49. Wess, J., K. Nakajima & S. Jain. 2013. Novel designer receptors to probe GPCR signaling and physiology. *Trends Pharmacol. Sci.* **34:** 385–392.
50. Hilário, M.R.F. & R.M. Costa. 2008. High on habits. *Front. Neurosci.* **2:** 208–217.
51. Koob, G.F. 2008. A role for brain stress systems in addiction. *Neuron* **59:** 11–34.
52. George, O. & G.F. Koob. 2013. Control of craving by the prefrontal cortex. *Proc. Natl. Acad. Sci. USA* **110:** 4165–4166.
53. Merol Pich, E.L.M., M. Yeganeh, F. Rodriguez de Fonseca, *et al.* 1995. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J. Neurosci.* **15:** 5439–5447.
54. Richter, R.M. & F. Weiss. 1999. *In vivo* CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. *Synapse* **32:** 254–261.
55. Rodríguez de Fonseca, F., M.R. Carrera, M. Navarro, *et al.* 1997. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* **276:** 2050–2054.
56. Weiss, F., R. Ciccocioppo, L.H. Parsons, *et al.* 2001. Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. *Ann. N.Y. Acad. Sci.* **937:** 1–26.
57. George, O., S. Ghozland, M.R. Azar, *et al.* 2007. CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. *Proc. Natl. Acad. Sci. USA* **104:** 17198–17203.
58. Rassnick, S., S.C. Heinrichs, K.T. Britton & G.F. Koob. 1993. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res.* **605:** 25–32.
59. Overstreet, D.H., D.J. Knapp & G.R. Breese. 2004. Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF1 receptors. *Pharmacol. Biochem. Behav.* **77:** 405–413.
60. Baldwin, H.A., S. Rassnick, J. Rivier, *et al.* 1991. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. *Psychopharmacology (Berl)* **103:** 227–232.
61. Funk, C.K., L.E. O'Dell, E.F. Crawford & G.F. Koob. 2006. Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. *J. Neurosci.* **26:** 11324–11332.
62. Koob, G.F. & M. Le Moal. 2001. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* **24:** 97–129.
63. Roberto, M., M.T. Cruz, N.W. Gilpin, *et al.* 2010. Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. *Biol. Psychiatry* **67:** 831–839.
64. Vendruscolo, L.F., E. Barbier, J.E. Schlosburg, *et al.* 2012. Corticosteroid-dependent plasticity mediates compulsive alcohol drinking in rats. *J. Neurosci.* **32:** 7563–7571.
65. Vendruscolo, L.F., D. Estey, V. Goodell, *et al.* 2015. Glucocorticoid receptor antagonism decreases alcohol seeking in alcohol-dependent individuals. *J. Clin. Invest.* **125:** 3193–3197.
66. Nestler, E.J. 2001. Molecular basis of long-term plasticity underlying addiction. *Nat. Rev. Neurosci.* **2:** 119–128.

67. Spanagel, R., A. Herz & T.S. Shippenberg. 1990. The effects of opioid peptides on dopamine release in the nucleus accumbens: an *in vivo* microdialysis study. *J. Neurochem.* **55:** 1734–1740.
68. Walker, B.M., E.P. Zorrilla, G.F. Koob. 2011. Systemic κ-opioid receptor antagonism by nor-binaltorphimine reduces dependence-induced excessive alcohol self-administration in rats. *Addict. Biol.* **16:** 116–119.
69. Nealey, K.A., A.W. Smith, S.M. Davis, *et al.* 2011. κ-opioid receptors are implicated in the increased potency of intra-accumbens nalmefene in ethanol-dependent rats. *Neuropharmacology* **61:** 35–42.
70. Gipson, C.D., K.J. Reissner, Y.M. Kupchik, *et al.* 2013. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proc. Natl. Acad. Sci. USA* **110:** 9124–9129.
71. Scofield, M.D., H. Li, B.M. Siemsen, *et al.* 2016. Cocaine self-administration and extinction leads to reduced glial fibrillary acidic protein expression and morphometric features of astrocytes in the nucleus accumbens core. *Biol. Psychiatry* **80:** 207–215.
72. Smith, A.C.W., Y.M. Kupchik, M.D. Scofield, *et al.* 2014. Synaptic plasticity mediating cocaine relapse requires matrix metalloproteinases. *Nat. Neurosci.* **17:** 1655–1657.
73. Wiggins, A., R.J. Smith, H.-W. Shen & P.W. Kalivas. 2011. Integrins modulate relapse to cocaine-seeking. *J. Neurosci.* **31:** 16177–16184.
74. Knackstedt, L.A., R.I. Melendez & P.W. Kalivas. 2010. Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. *Biol. Psychiatry* **67:** 81–84.
75. Shen, H., M.D. Scofield, H. Boger, *et al.* 2014. Synaptic glutamate spillover due to impaired glutamate uptake mediates heroin relapse. *J. Neurosci.* **34:** 5649–5657.
76. Sari, Y. & S.N. Sreemantula. 2012. Neuroimmunophilin GPI-1046 reduces ethanol consumption in part through activation of GLT1 in alcohol-preferring rats. *Neuroscience* **227:** 327–335.
77. McFarland, K., C.C. Lapish & P.W. Kalivas. 2003. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.* **23:** 3531–3537.
78. Gass, J.T., C.M. Sinclair, R.M. Cleva, *et al.* 2011. Alcohol-seeking behavior is associated with increased glutamate transmission in basolateral amygdala and nucleus accumbens as measured by glutamate-oxidase-coated biosensors. *Addict. Biol.* **16:** 215–228.
79. Parsegian, A. & R.E. See. 2014. Dysregulation of dopamine and glutamate release in the prefrontal cortex and nucleus accumbens following methamphetamine self-administration and during reinstatement in rats. *Neuropsychopharmacology* **39:** 811–822.
80. Sari, Y., K.D. Smith, P.K. Ali & G.V. Rebec. 2009. Upregulation of GLT1 attenuates cue-induced reinstatement of cocaine-seeking behavior in rats. *J. Neurosci.* **29:** 9239–9243.
81. Moussawi, K., A. Pacchioni, M. Moran, *et al.* 2009. N-acetylcysteine reverses cocaine-induced metaplasticity. *Nat. Neurosci.* **12:** 182–189.
82. Moussawi, K., W. Zhou, H. Shen, *et al.* 2011. Reversing cocaine-induced synaptic potentiation provides enduring protection from relapse. *Proc. Natl. Acad. Sci. USA* **108:** 385–390.
83. Reissner, K.J., R.M. Brown, S. Spencer, *et al.* 2014. Chronic administration of the methylxanthine propentofylline impairs reinstatement to cocaine by a GLT-1-dependent mechanism. *Neuropsychopharmacology* **39:** 499–506.
84. Knackstedt, L.A., S. LaRowe, P. Mardikian, *et al.* 2009. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biol. Psychiatry* **65:** 841–845.
85. Ramirez-Niño, A.M., M.S. D'Souza & A. Markou. 2013. N-acetylcysteine decreased nicotine self-administration and cue-induced reinstatement of nicotine seeking in rats: comparison with the effects of N-acetylcysteine on food responding and food seeking. *Psychopharmacology (Berl)* **225:** 473–482.
86. Sari, Y., M. Sakai, J.M. Weedman, *et al.* 2011. Ceftriaxone, a beta-lactam antibiotic, reduces ethanol consumption in alcohol-preferring rats. *Alcohol Alcohol* **46:** 239–246.
87. Grant, J.E., S.W. Kim & B.L. Odlaug. 2007. N-acetyl cysteine, a glutamate-modulating agent, in the treatment of pathological gambling: a pilot study. *Biol. Psychiatry* **62:** 652–657.
88. Grant, J.E., B.L. Odlaug & S.W. Kim. 2009. N-acetylcysteine, a glutamate modulator, in the treatment of trichotillomania: a double-blind, placebo-controlled study. *Arch. Gen. Psychiatry* **66:** 756–763.
89. Afshar, H., H. Roohafza, H. Mohammad-Beigi, *et al.* 2012. N-acetylcysteine add-on treatment in refractory obsessive-compulsive disorder: a randomized, double-blind, placebo-controlled trial. *J. Clin. Psychopharmacol.* **32:** 797–803.
90. Berk, M., D.L. Copolov, O. Dean, *et al.* 2008. N-acetyl cysteine for depressive symptoms in bipolar disorder—a double-blind randomized placebo-controlled trial. *Biol. Psychiatry* **64:** 468–475.
91. Berk, M., O.M. Dean, S.M. Cotton, *et al.* 2014. The efficacy of adjunctive N-acetylcysteine in major depressive disorder: a double-blind, randomized, placebo-controlled trial. *J. Clin. Psychiatry* **75:** 628–636.
92. Bloch, M.H., K.E. Panza, J.E. Grant, *et al.* 2013. N-acetylcysteine in the treatment of pediatric trichotillomania: a randomized, double-blind, placebo-controlled add-on trial. *J. Am. Acad. Child. Adolesc. Psychiatry* **52:** 231–240.
93. Grant, J.E., B.L. Odlaug & S.W. Kim. 2010. A double-blind, placebo-controlled study of N-acetyl cysteine plus naltrexone for methamphetamine dependence. *Eur. Neuropsychopharmacol.* **20:** 823–828.
94. Back, S.E., J.L. McCauley, K.J. Korte, *et al.* 2016. A double-blind, randomized, controlled pilot trial of N-acetylcysteine in veterans with posttraumatic stress disorder and substance use disorders. *J. Clin. Psychiatry* **77:** e1439–e1446.
95. Andrews, P.W., A. Bharwani, K.R. Lee, *et al.* 2015. Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neurosci. Biobehav. Rev.* **51:** 164–188.
96. Thibaut, F. 2017. Anxiety disorders: a review of current literature. *Dialogues Clin. Neurosci.* **19:** 87–88.

97. Lilly USA, LLC. 2017. Prozac [prescribing information].
98. Pfizer, Inc. 2018. Zoloft [prescribing information].
99. Burghardt, N.S. & E.P. Bauer. 2013. Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: implications for underlying fear circuits. *Neuroscience* **247:** 253–272.
100. Krystal, J.H., E. Webb, N. Cooney, *et al.* 1994. Specificity of ethanollike effects elicited by serotonergic and noradrenergic mechanisms. *Arch. Gen. Psychiatry* **51:** 898–911.
101. Umhau, J.C., M.L. Schwandt, J. Usala, *et al.* 2011. Pharmacologically induced alcohol craving in treatment seeking alcoholics correlates with alcoholism severity, but is insensitive to acamprosate. *Neuropsychopharmacology* **36:** 1178–1186.
102. Kranzler, H.R., R. Feinn, S. Armeli & H. Tennen. 2012. Comparison of alcoholism subtypes as moderators of the response to sertraline treatment. *Alcohol. Clin. Exp. Res.* **36:** 509–516.
103. Kranzler, H.R., S. Armeli, H. Tennen, *et al.* 2011. A double-blind, randomized trial of sertraline for alcohol dependence: moderation by age of onset [corrected] and 5-hydroxytryptamine transporter-linked promoter region genotype. *J. Clin. Psychopharmacol.* **31:** 22–30.
104. Lowery-Gionta, E.G., C.A. Marcinkiewcz & T.L. Kash. 2015. Functional alterations in the dorsal raphe nucleus following acute and chronic ethanol exposure. *Neuropsychopharmacology* **40:** 590–600.
105. Marcinkiewcz, C.A., C.E. Dorrier, A.J. Lopez & T.L. Kash. 2015. Ethanol induced adaptations in 5-HT2c receptor signaling in the bed nucleus of the stria terminalis: implications for anxiety during ethanol withdrawal. *Neuropharmacology* **89:** 157–167.
106. Burghardt, N.S., D.E.A. Bush, B.S. McEwen & J.E. LeDoux. 2007. Acute selective serotonin reuptake inhibitors increase conditioned fear expression: blockade with a 5-HT(2C) receptor antagonist. *Biol. Psychiatry* **62:** 1111–1118.
107. Marcinkiewcz, C.A., C.M. Mazzone, G. D'Agostino, *et al.* 2016. Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. *Nature* **537:** 97–101.
108. Lopez, M.F., T.L. Doremus-Fitzwater & H.C. Becker. 2011. Chronic social isolation and chronic variable stress during early development induce later elevated ethanol intake in adult C57BL/6J mice. *Alcohol* **45:** 355–364.
109. Lopez, M.F. & K. Laber. 2015. Impact of social isolation and enriched environment during adolescence on voluntary ethanol intake and anxiety in C57BL/6J mice. *Physiol. Behav.* **148:** 151–156.
110. Roeckner, A.R., A. Bowling & T.R. Butler. 2017. Chronic social instability increases anxiety-like behavior and ethanol preference in male Long Evans rats. *Physiol. Behav.* **173:** 179–187.
111. Russo, S.J., D.M. Dietz, D. Dumitriu, *et al.* 2010. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* **33:** 267–276.
112. Cahill, M.E., R.C. Bagot, A.M. Gancarz, *et al.* 2016. Bidirectional synaptic structural plasticity after chronic cocaine administration occurs through Rap1 small GTPase signaling. *Neuron* **89:** 566–582.
113. Egervari, G., J. Landry, J. Callens, *et al.* 2017. Striatal H3K27 acetylation linked to glutamatergic gene dysregulation in human heroin abusers holds promise as therapeutic target. *Biol. Psychiatry* **81:** 585–594.
114. Gallenkamp, D., K.A. Gelato, B. Haendler & H. Weinmann. 2014. Bromodomains and their pharmacological inhibitors. *ChemMedChem* **9:** 438–464.
115. Benowitz, N.L. & P. Jacob. 1994. Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clin. Pharmacol. Ther.* **56:** 483–493.
116. Messina, E.S., R.F. Tyndale & E.M. Sellers. 1997. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. *J. Pharmacol. Exp. Ther.* **282:** 1608–1614.
117. Dempsey, D., P. Tutka, P. Jacob, *et al.* 2004. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin. Pharmacol. Ther.* **76:** 64–72.
118. Nakajima, M., T. Yamamoto, K. Nunoya, *et al.* 1996. Role of human cytochrome P4502A6 in C-oxidation of nicotine. *Drug Metab. Dispos.* **24:** 1212–1217.
119. Rao, Y., E. Hoffmann, M. Zia, *et al.* 2000. Duplications and defects in the CYP2A6 gene: identification, genotyping, and *in vivo* effects on smoking. *Mol. Pharmacol.* **58:** 747–755.
120. Strasser, A.A., V. Malaiyandi, E. Hoffmann, *et al.* 2007. An association of CYP2A6 genotype and smoking topography. *Nicotine Tob. Res.* **9:** 511–518.
121. Chenoweth, M.J., J. O'Loughlin, M.-P. Sylvestre & R.F. Tyndale. 2013. CYP2A6 slow nicotine metabolism is associated with increased quitting by adolescent smokers. *Pharmacogenet. Genomics* **23:** 232–235.
122. Ray, R., R.F. Tyndale & C. Lerman. 2009. Nicotine dependence pharmacogenetics: role of genetic variation in nicotine-metabolizing enzymes. *J. Neurogenet.* **23:** 252–261.
123. Patterson, F., R.A. Schnoll, E.P. Wileyto, *et al.* 2008. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. *Clin. Pharmacol. Ther.* **84:** 320–325.
124. Zhu, A.Z.X., L.S. Cox, N. Nollen, *et al.* 2012. CYP2B6 and bupropion's smoking-cessation pharmacology: the role of hydroxybupropion. *Clin. Pharmacol. Ther.* **92:** 771–777.
125. Lerman, C., R. Tyndale, F. Patterson, *et al.* 2006. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clin. Pharmacol. Ther.* **79:** 600–608.
126. Lerman, C., C. Jepson, E.P. Wileyto, *et al.* 2010. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. *Clin. Pharmacol. Ther.* **87:** 553–557.
127. Lerman, C., R.A. Schnoll, L.W. Hawk, *et al.* 2015. Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial. *Lancet Respir. Med.* **3:** 131–138.
128. Tang, D.W., B. Hello, M. Mroziewicz, *et al.* 2012. Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI. *Neuroimage* **60:** 2136–2143.
129. Bava, S., R. Thayer, J. Jacobus, *et al.* 2010. Longitudinal characterization of white matter maturation during adolescence. *Brain Res.* **1327:** 38–46.

130. Galvan, A., T.A. Hare, C.E. Parra, *et al.* 2006. Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. *J. Neurosci.* **26:** 6885–6892.
131. NIAAA Publications.
132. Grant, B.F. & D.A. Dawson. 1997. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J. Subst. Abuse* **9:** 103–110.
133. Hingson, R.W., T. Heeren & M.R. Winter. 2006. Age at drinking onset and alcohol dependence: age at onset, duration, and severity. *Arch. Pediatr. Adolesc. Med.* **160:** 739–746.
134. Lisdahl, K.M., R. Thayer, L.M. Squeglia, *et al.* 2013. Recent binge drinking predicts smaller cerebellar volumes in adolescents. *Psychiatry Res.* **211:** 17–23.
135. Brown, S.A., S.F. Tapert, E. Granholm & D.C. Delis. 2000. Neurocognitive functioning of adolescents: effects of protracted alcohol use. *Alcohol. Clin. Exp. Res.* **24:** 164–171.
136. Squeglia, L.M., A.D. Spadoni, M.A. Infante, *et al.* 2009. Initiating moderate to heavy alcohol use predicts changes in neuropsychological functioning for adolescent girls and boys. *Psychol. Addict. Behav.* **23:** 715–722.
137. Nguyen-Louie, T.T., A. Tracas, L.M. Squeglia, *et al.* 2016. Learning and memory in adolescent moderate, binge, and extreme-binge drinkers. *Alcohol. Clin. Exp. Res.* **40:** 1895–1904.
138. Squeglia, L.M., S.F. Tapert, E.V. Sullivan, *et al.* 2015. Brain development in heavy-drinking adolescents. *Am. J. Psychiatry* **172:** 531–542.
139. Squeglia, L.M., C. Pulido, R.R. Wetherill, *et al.* 2012. Brain response to working memory over three years of adolescence: influence of initiating heavy drinking. *J. Stud. Alcohol Drugs* **73:** 749–760.
140. Squeglia, L.M., T.M. Ball, J. Jacobus, *et al.* 2017. Neural predictors of initiating alcohol use during adolescence. *Am. J. Psychiatry* **174:** 172–185.
141. Tapert, S.F., E.H. Cheung, G.G. Brown, *et al.* 2003. Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Arch. Gen. Psychiatry* **60:** 727–735.
142. Brumback, T., L.M. Squeglia, J. Jacobus, *et al.* 2015. Adolescent heavy drinkers' amplified brain responses to alcohol cues decrease over one month of abstinence. *Addict. Behav.* **46:** 45–52.
143. Brown, S.A., T. Brumback, K. Tomlinson, *et al.* 2015. The National Consortium on Alcohol and NeuroDevelopment in Adolescence (NCANDA): a multisite study of adolescent development and substance use. *J. Stud. Alcohol Drugs* **76:** 895–908.
144. Volkow, N.D., G.F. Koob, R.T. Croyle, *et al.* 2018. The conception of the ABCD study: from substance use to a broad NIH collaboration. *Dev. Cogn. Neurosci.* 32: 4–7.
145. National Institutes of Health. 2018. NIH releases first dataset from unprecedented study of adolescent brain development. National Institutes of Health. https://www.nih.gov/news-events/news-releases/nih-releases-first-dataset-unprecedented-study-adolescent-brain-development
146. Wood, K.E., L.L. Sinclair, C.D. Rysgaard, *et al.* 2014. Retrospective analysis of the diagnostic yield of newborn drug testing. *BMC Pregnancy Childbirth* **14:** 250.
147. Wang, X., D. Dow-Edwards, V. Anderson, *et al.* 2004. *In utero* marijuana exposure associated with abnormal amygdala dopamine D2 gene expression in the human fetus. *Biol. Psychiatry* **56:** 909–915.
148. DiNieri, J.A., X. Wang, H. Szutorisz, *et al.* 2011. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. *Biol. Psychiatry* **70:** 763–769.
149. Szutorisz, H., J.A. DiNieri, E. Sweet, *et al.* 2014. Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. *Neuropsychopharmacology* **39:** 1315–1323.
150. Johnston, L.D., R.A. Miech, P.M. O'Malley, *et al.* 2018. *Key Findings on Adolescent Drug Use: 2017 Overview*. Ann Arbor, MI: Institute for Social Research, the University of Michigan: The National Institute on Drug Abuse at the National Institutes of Health.
151. Gruber, S.A., K.A. Sagar, M.K. Dahlgren, *et al.* 2012. Age of onset of marijuana use and executive function. *Psychol. Addict. Behav.* **26:** 496–506.
152. Gruber, S.A., M.K. Dahlgren, K.A. Sagar, *et al.* 2014. Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. *Psychopharmacology (Berl)* **231:** 1455–1465.
153. Meier, M.H., A. Caspi, A. Ambler, *et al.* 2012. Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proc. Natl. Acad. Sci. USA* **109:** E2657–E2664.
154. Grant, J.D., J.F. Scherrer, M.T. Lynskey, *et al.* 2012. Associations of alcohol, nicotine, cannabis and drug use/dependence with educational attainment: evidence from cotwin-control analyses. *Alcohol. Clin. Exp. Res.* **36:** 1412–1420.
155. Verweij, K.J.H., A.C. Huizink, A. Agrawal, *et al.* 2013. Is the relationship between early-onset cannabis use and educational attainment causal or due to common liability? *Drug Alcohol Depend.* **133:** 580–586.
156. Weiland, B.J., R.E. Thayer, B.E. Depue, *et al.* 2015. Daily marijuana use is not associated with brain morphometric measures in adolescents or adults. *J. Neurosci.* **35:** 1505–1512.
157. Jamal, A., A. Gentzke, S.S. Hu, *et al.* 2017. Tobacco use among middle and high school students—United States, 2011–2016. *MMWR Morb. Mortal. Wkly. Rep.* **66:** 597–603.
158. Singh, T., S. Kennedy, K. Marynak, *et al.* 2016. Characteristics of electronic cigarette use among middle and high school students—United States, 2015. *MMWR Morb. Mortal. Wkly. Rep.* **65:** 1425–1429.
159. Food and Drug Administration, HHS. 2016. Deeming tobacco products to be subject to the Federal Food, Drug, and Cosmetic Act, as amended by the Family Smoking Prevention and Tobacco Control Act; restrictions on the sale and distribution of tobacco products and required warning statements for tobacco products. *Fed. Regis.* **81:** 28973–29106.

160. Wheeler, L. 2018. FDA faces new lawsuits over e-cigarette rule. *The Hill* January 30, 2018.
161. Wheeler, L. 2016. Company files first lawsuit against the FDA's e-cigarette rule. *The Hill* May 10, 2016.
162. Glasser, A.M., C.O. Cobb, L. Teplitskaya, *et al.* 2015. Electronic nicotine delivery devices, and their impact on health and patterns of tobacco use: a systematic review protocol. *BMJ Open* **5:** e007688.
163. Duke, J.C., J.A. Allen, M.E. Eggers, *et al.* 2016. Exploring differences in youth perceptions of the effectiveness of electronic cigarette television advertisements. *Nicotine Tob. Res.* **18:** 1382–1386.
164. Duke, J.C., Y.O. Lee, A.E. Kim, *et al.* 2014. Exposure to electronic cigarette television advertisements among youth and young adults. *Pediatrics* **134:** e29–e36.
165. Owotomo, O., J. Maslowsky & A. Loukas. 2018. Perceptions of the harm and addictiveness of conventional cigarette smoking among adolescent e-cigarette users. *J. Adolesc. Health* **62:** 87–93.
166. Vansickel, A.R., C.O. Cobb, M.F. Weaver & T.E. Eissenberg. 2010. A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and cardiovascular and subjective effects. *Cancer Epidemiol. Biomark. Prev.* **19:** 1945–1953.
167. Yan, X.S. & C. D'Ruiz. 2015. Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regul. Toxicol. Pharmacol.* **71:** 24–34.
168. Farsalinos, K.E., A. Spyrou, K. Tsimopoulou, *et al.* 2014. Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Sci. Rep.* **4:** 4133.
169. Ramôa, C.P., M.M. Hiler, T.R. Spindle, *et al.* 2016. Electronic cigarette nicotine delivery can exceed that of combustible cigarettes: a preliminary report. *Tob. Control* **25:** e6–e9.
170. Talih, S., Z. Balhas, T. Eissenberg, *et al.* 2015. Effects of user puff topography, device voltage, and liquid nicotine concentration on electronic cigarette nicotine yield: measurements and model predictions. *Nicotine Tob. Res.* **17:** 150–157.
171. Spindle, T.R., A.B. Breland, N.V. Karaoghlanian, *et al.* 2015. Preliminary results of an examination of electronic cigarette user puff topography: the effect of a mouthpiece-based topography measurement device on plasma nicotine and subjective effects. *Nicotine Tob. Res.* **17:** 142–149.
172. Weder, N. & J. Kaufman. 2011. Critical periods revisited: implications for intervention with traumatized children. *J. Am. Acad. Child Adolesc. Psychiatry* **50:** 1087–1089.
173. Davidson, R.J., J. Kabat-Zinn, J. Schumacher, *et al.* 2003. Alterations in brain and immune function produced by mindfulness meditation. *Psychosom. Med.* **65:** 564–570.
174. Tang, Y.-Y. & M.I. Posner. 2009. Attention training and attention state training. *Trends Cogn. Sci.* **13:** 222–227.
175. Kerr, C.E., S.R. Jones, Q. Wan, *et al.* 2011. Effects of mindfulness meditation training on anticipatory alpha modulation in primary somatosensory cortex. *Brain Res. Bull.* **85:** 96–103.
176. Kilpatrick, L.A., B.Y. Suyenobu, S.R. Smith, *et al.* 2011. Impact of mindfulness-based stress reduction training on intrinsic brain connectivity. *Neuroimage* **56:** 290–298.
177. Fishbein, D.H., C. Domitrovich, J. Williams, *et al.* 2016. Short-term intervention effects of the PATHS curriculum in young low-income children: capitalizing on plasticity. *J. Prim. Prev.* **37:** 493–511.
178. Piehler, T.F., M.L. Bloomquist, G.J. August, *et al.* 2014. Executive functioning as a mediator of conduct problems prevention in children of homeless families residing in temporary supportive housing: a parallel process latent growth modeling approach. *J. Abnorm. Child Psychol.* **42:** 681–692.
179. Bierman, K.L., R.L. Nix, M.T. Greenberg, *et al.* 2008. Executive functions and school readiness intervention: impact, moderation, and mediation in the Head Start REDI program. *Dev. Psychopathol.* **20:** 821–843.
180. Fisher, P.A., M. Stoolmiller, M.R. Gunnar & B.O. Burraston. 2007. Effects of a therapeutic intervention for foster preschoolers on diurnal cortisol activity. *Psychoneuroendocrinology* **32:** 892–905.
181. Yizhar, O., L.E. Fenno, T.J. Davidson, *et al.* 2011. Optogenetics in neural systems. *Neuron* **71:** 9–34.
182. Kim, T., J.G. McCall, Y.H. Jung, *et al.* 2013. Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. *Science* **340:** 211–216.
183. Jeong, J.-W., J.G. McCall, G. Shin, *et al.* 2015. Wireless optofluidic systems for programmable *in vivo* pharmacology and optogenetics. *Cell* **162:** 662–674.
184. Pascoli, V., M. Turiault & C. Lüscher. 2011. Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature* **481:** 71–75.
185. Mameli, M., B. Balland, R. Luján & C. Lüscher. 2007. Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science* **317:** 530–533.
186. Mameli, M., B. Halbout, C. Creton, *et al.* 2009. Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc. *Nat. Neurosci.* **12:** 1036–1041.
187. Creed, M., V.J. Pascoli & C. Lüscher. 2015. Addiction therapy. Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology. *Science* **347:** 659–664.
188. Koob, G., M.J. Hicks, S. Wee, *et al.* 2011. Anti-cocaine vaccine based on coupling a cocaine analog to a disrupted adenovirus. *CNS Neurol. Disord. Drug Targets* **10:** 899–904.
189. Hicks, M.J., S.M. Kaminsky, B.P. De, *et al.* 2014. Fate of systemically administered cocaine in nonhuman primates treated with the dAd5GNE anti-cocaine vaccine. *Hum. Gene Ther. Clin. Dev.*25**:** 40–49.
190. Wee, S., M.J. Hicks, B.P. De, *et al.* 2012. Novel cocaine vaccine linked to a disrupted adenovirus gene transfer vector blocks cocaine psychostimulant and reinforcing effects. *Neuropsychopharmacology* **37:** 1083–1091.
191. Evans, S.M., R.W. Foltin, M.J. Hicks, *et al.* 2016. Efficacy of an adenovirus-based anti-cocaine vaccine to reduce cocaine self-administration and reacqusition using a choice procedure in rhesus macaques. *Pharmacol. Biochem. Behav.* **150–151:** 76–86.

192. Safety study of a disrupted adenovirus (Ad) serotype cocaine vaccine for cocaine-dependent individuals—full text view—ClinicalTrials.gov.
193. Rosenberg, J.B., M.J. Hicks, B.P. De, *et al.* 2012. AAVrh.10-mediated expression of an anti-cocaine antibody mediates persistent passive immunization that suppresses cocaine-induced behavior. *Hum. Gene Ther.* **23:** 451–459.
194. Substance Abuse and Mental Health Services Administration. 2014. Results from the 2013 National Survey on Drug Use and Health: summary of national findings. Rockville, MD: Substance Abuse and Mental Health Services Administration.
195. Hedegaard, H., M. Warner & A.M. Minino. 2017. *Drug Overdose Deaths in the United States, 1999–2016.* Hyattsville, MD: National Center for Health Statistics.
196. CDC, National Center for Health Statistics. Wide-ranging online data for epidemiologic research (WONDER). Atlanta, GA: CDC, National Center for Health Statistics.
197. Volkow, N.D., T.R. Frieden, P.S. Hyde & S.S. Cha. 2014. Medication-assisted therapies—tackling the opioid-overdose epidemic. *N. Engl. J. Med.* **370:** 2063–2066.
198. Gordon, M.S., T.W. Kinlock, R.P. Schwartz & K.E. O'Grady. 2008. A randomized clinical trial of methadone maintenance for prisoners: findings at 6 months post-release. *Addiction* **103:** 1333–1342.
199. Ball, J.C. & A. Ross. 1991. *The Effectiveness of Methadone Maintenance Treatment.* 1st ed. New York: Springer-Verlag.
200. Fiellin, D.A., L. Weiss, M. Botsko, *et al.* 2011. Drug treatment outcomes among HIV-infected opioid-dependent patients receiving buprenorphine/naloxone. *J. Acquir. Immune Defic. Syndr.* **56**(Suppl. 1): S33–S38.
201. Hser, Y.-I., A.J. Saxon, D. Huang, *et al.* 2014. Treatment retention among patients randomized to buprenorphine/naloxone compared to methadone in a multi-site trial. *Addiction* **109:** 79–87.
202. Baser, O., M. Chalk, D.A. Fiellin & D.R. Gastfriend. 2011. Cost and utilization outcomes of opioid-dependence treatments. *Am. J. Manag. Care* **17**(Suppl. 8): S235–S248.
203. Weiss, R.D., J.S. Potter, D.A. Fiellin, *et al.* 2011. Adjunctive counseling during brief and extended buprenorphine-naloxone treatment for prescription opioid dependence: a 2-phase randomized controlled trial. *Arch. Gen. Psychiatry* **68:** 1238–1246.
204. Mattick, R.P., C. Breen, J. Kimber & M. Davoli. 2014. Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. *Cochrane Database Syst. Rev.* CD002207.
205. Rapeli, P., R. Kivisaari, T. Autti, *et al.* 2006. Cognitive function during early abstinence from opioid dependence: a comparison to age, gender, and verbal intelligence matched controls. *BMC Psychiatry* **6:** 9.
206. Mintzer, M.Z. & M.L. Stitzer. 2002. Cognitive impairment in methadone maintenance patients. *Drug Alcohol Depend.* **67:** 41–51.
207. Curran, H.V., J. Kleckham, J. Bearn, *et al.* 2001. Effects of methadone on cognition, mood and craving in detoxifying opiate addicts: a dose-response study. *Psychopharmacology (Berl)* **154:** 153–160.
208. Pirastu, R., R. Fais, M. Messina, *et al.* 2006. Impaired decision-making in opiate-dependent subjects: effect of pharmacological therapies. *Drug Alcohol Depend.* **83:** 163–168.
209. Soyka, M., M. Horak, S. Dittert & S. Kagerer. 2001. Less driving impairment on buprenorphine than methadone in drug-dependent patients? *J. Neuropsychiatry Clin. Neurosci.* **13:** 527–528.
210. Minozzi, S., L. Amato, S. Vecchi, *et al.* 2011. Oral naltrexone maintenance treatment for opioid dependence. *Cochrane Database Syst. Rev.* CD001333.
211. Gibson, A.E. & L.J. Degenhardt. 2007. Mortality related to pharmacotherapies for opioid dependence: a comparative analysis of coronial records. *Drug Alcohol Rev.* **26:** 405–410.
212. Langleben, D.D., K. Ruparel, I. Elman, *et al.* 2014. Extended-release naltrexone modulates brain response to drug cues in abstinent heroin-dependent patients. *Addict. Biol.* **19:** 262–271.
213. Krupitsky, E., E.V. Nunes, W. Ling, *et al.* 2011. Injectable extended-release naltrexone for opioid dependence: a double-blind, placebo-controlled, multicentre randomised trial. *Lancet* **377:** 1506–1513.
214. Coviello, D.M., J.W. Cornish, K.G. Lynch, *et al.* 2012. A multisite pilot study of extended-release injectable naltrexone treatment for previously opioid-dependent parolees and probationers. *Subst. Abuse* **33:** 48–59.
215. Lee, J.D., P.D. Friedmann, T.W. Kinlock, *et al.* 2016. Extended-release naltrexone to prevent opioid relapse in criminal justice offenders. *N. Engl. J. Med.* **374:** 1232–1242.
216. MacKillop, J., M.T. Amlung, L.R. Few, *et al.* 2011. Delayed reward discounting and addictive behavior: a meta-analysis. *Psychopharmacology (Berl)* **216:** 305–321.
217. Landes, R.D., D.R. Christensen & W.K. Bickel. 2012. Delay discounting decreases in those completing treatment for opioid dependence. *Exp. Clin. Psychopharmacol.* **20:** 302–309.
218. Nicosia, N., B. Kilmer & P. Heaton. 2016. Can a criminal justice alcohol abstention programme with swift, certain, and modest sanctions (24/7 Sobriety) reduce population mortality? A retrospective observational study. *Lancet Psychiatry* **3:** 226–232.
219. Migoya, D. & R. Baca. 2016. Denver's pot businesses mostly in low-income, minority neighborhoods. *Denver Post* January 2, 2016.
220. LaVeist, T.A. & J.M. Wallace. 2000. Health risk and inequitable distribution of liquor stores in African American neighborhood. *Soc. Sci. Med.* **51:** 613–617.
221. Colorado Department of Public Safety, Division of Criminal Justice. A Report Pursuant to Senate Bill 13-283. 2016. Marijuana legalization in Colorado: early findings. Denver, CO.
222. Wise, R.A. & G.F. Koob. 2014. The development and maintenance of drug addiction. *Neuropsychopharmacology* **39:** 254–262.