Published in final edited form as:

Expert Opin Drug Discov. 2014 November ; 9(11): 1319–1331. doi:10.1517/17460441.2014.956721.

Current approaches for the discovery of drugs that deter substance and drug abuse

Adam Yasgar¹ and Anton Simeonov^{1,*}

¹NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, USA

Abstract

Introduction—Much has been presented and debated on the topic of drug abuse and its multidimensional nature, including the role of society and its customs and laws, economical factors, and the magnitude and nature of the burden. Given the complex nature of the receptors and pathways implicated in regulation of the cognitive and behavioral processes associated with addiction, a large number of molecular targets have been interrogated during recent years to discover starting points for development of small molecule interventions.

Areas covered—This review describes recent developments in the field of early drug discovery for drug abuse interventions, with a special emphasis on advances published during the 2012–2014 period.

Expert Opinion—Technologically, the processes/platforms utilized in drug abuse drug discovery are nearly identical to those used in the other disease areas. A key complicating factor in drug abuse research is the enormous biological complexity surrounding the brain processes involved and the associated difficulty in finding “good” targets and achieving exquisite selectivity of treatment agents. While tremendous progress has been made during recent years to use the power of high-throughput technologies to discover proof-of-principle molecules for many new targets, next-generation models will be especially important in this field; examples include seeking advantageous drug-drug combinations, use of automated whole-animal behavioral screening systems, advancing our understanding of the role of epigenetics in drug addiction, and the employment of organoid-level 3D test platforms (also referred to as tissue-chip or organs-on-chip).

Keywords

drug abuse; addiction; substance abuse; drugs of abuse; high-throughput screening; receptor agonist; combination therapy

1. Introduction

The use of xenobiotics as remedies for illness has enabled society to combat diseases that for centuries were thought to be not curable or treatable. Unfortunately, not all medicines are benevolent, as the ability to cure often comes with some serious side-, or off-target effects,

*To whom correspondence should be addressed, asimeono@mail.nih.gov, Phone: 301-217-5721, Fax: 301-217-5736.

one of them being addiction or abuse. Substance abuse[1], also known as drug abuse (other terms being used in this context are drugs of abuse, addiction, substance abuse, substance disorders, substance use disorders) is a patterned use of a substance (drug) in which the user consumes the substance in amounts or with methods which are harmful to themselves or others. Drug addiction is defined as the continued compulsive use of drugs despite adverse health or social consequences. These consequences are far from minor: in 2008, The National Institute on Drug Abuse (NIDA) reported that an estimated \$559 billion/year is spent on substance abuse and addiction in the form of health care costs, productivity loss, crime, incarceration, and drug enforcement [2,3].

Substance abuse presents a long-term vexing problem for society and as such the topic is well reviewed in the scientific literature [4-10], with recurring themes being prevention, identification, treatment, and the social and pharmacological ways to diagnose and treat each. Developing agents that treat substance abuse employs a process nearly identical to that used in the traditional-disease therapeutic discovery and development pipeline: initial research into the underlying biology and identification of a suitable molecular target, followed by the associated steps of early discovery and optimization, next testing in animal models, and proceeding to human trials. After an introduction of a treatment modality, the final step of post-market surveillance aids in understanding the public's response and the effects of the new intervention [11-14]. This has typically been a reactionary process, with researchers and clinicians on the defensive. One of the most common treatments for substance/drug abuse has been, ironically, drugs, due to their ability for quick onset of action in eliminating the users' desire for said substance. But with typical drugs taking well over a decade to be developed, finding a quick solution, especially in response to a new emerging substance of abuse, has been very difficult[15]. With its ability to speed up the discovery of drug candidates and lower overall costs, high-throughput screening (HTS) plays an important role in the process [16].

The literature is rich in reports of molecular targets, pathways, and mechanisms thought to be associated with the action of drugs of abuse [6,17-21]. Typically, the substances of abuse exert their action by modulating the production and transmission of chemicals (for example, neurotransmitters such as serotonin) through the receptors they bind, which in turn leads to a dysregulation of the cellular signaling[6]. Modulating such receptors with new drugs offers a potential therapy at curbing abuse [22,23] and high-throughput screening (HTS)[24], with its ability to facilitate test thousands-to-millions of compounds, while also recently incorporating dose-response format [25], provides a welcome avenue for early drug discovery. Herein, we highlight the variety of assay platforms employed to interrogate targets implicated in substance abuse. In addition to examples of how HTS has been employed in the discovery of small molecule modulators, we also briefly cover the topics of biologics [26] and vaccines[27] as potential interventions for substance abuse.

2. High-throughput screening

HTS is made possible by the use of robotic equipment and automated liquid handlers that operate autonomously, combined with the ability to track samples and process the data accurately [28-30]. A key enabling factor is the density of samples that can be assayed, with

384-, 1,536-, and 3,456-well plate formats now a commonplace, allowing millions of samples to be tested in a week [28]. While often being characterized as a still-emerging approaching, recent analyses have shown that utilization of HTS in the discovery process has resulted in benefits, as judged by the generation of new molecular entities [16,31], highlighting the immediate impact of HTS on all therapeutic areas including the field of substance abuse [32] (Table 1).

2.1. Drug abuse target categories addressed via cell-based HTS

A factor that significantly complicates substance abuse research is the number of biological components involved: for example, a recent chemogenomics knowledgebase publication listed over 85 G-protein coupled receptors (GPCRs) alone thought to be involved in substance abuse, a daunting number [33]. Below, we provide limited examples on the recent utilization of high-throughput assays to discover cell-active modulators of several key target classes implicated in drug abuse/addiction (also see Table 2).

2.1.1. Opioids/Opiates—The κ -opioid receptor (KOR)-dynorphin system has been implicated in the control of affect, cognition, and motivation, and is thought to be dysregulated in mood and psychotic disorders, as well as in various phases of opioid dependence [34,35]. Using miniaturized assays based on a genetically engineered firefly luciferase cyclic AMP (cAMP) biosensor, the Tango™ and bioluminescence resonance energy transfer (BRET) technologies, several KOR-selective ligand scaffolds were recently identified, displaying a range of signaling biases *in vitro* [36]. In another recent study, two classes of biased KOR agonists that potently activate G-protein coupling but weakly recruit arrestin 2 were discovered through a 96-well radiolabeled [³⁵S]GTP γ S and a 384-well GFP-labeled U2OS-hKOR- β arrestin2 cell methods, respectively[37].

2.1.2. Serotonin and Dopamine—During the past few decades evidence has steadily accumulated that 5-HT_{2C} receptor agonists alter various behaviors and underlying neurobiological systems relevant to drug abuse and addiction, supporting the possibility for use of selective 5-HT_{2C} agonists to treat nicotine and psychostimulant dependence [38]. Using a 96-well serotonin radiolabel assay, and a 384-well Cy3b-labeled fluorescence polarization (FP) binding assay, the small molecule 5a was identified as a high-affinity tracer ligand for assessing the binding affinity of novel ligands for 5HT_{2C} [39].

Transporters for dopamine, norepinephrine and serotonin (DAT, NET and SERT, respectively) represent established targets for many pharmacological agents that affect brain function, including antidepressants and psychostimulants. Using a novel neurotransmitter transporter uptake assay kit based on a 384-well FLIPR^{TETRA} cell transporter assay with a neurotransmitter loading dye, the authors demonstrated the ability to monitor the dynamic transport activity of DAT, NET and SERT by employing a fluorescent substrate that mimics the biogenic amine neurotransmitters. The labeled substrate is taken up by the cell through the specific transporters, resulting in increased fluorescence intensity, forming the basis of an assay amenable to high-throughput screening and compound characterization [40].

Dopamine D₂ receptors inhibit the activity of neurons on which they are expressed; thus, a decrease in D₂ receptor activity in indirect pathway neurons would take away a layer of

inhibition and would be expected to increase indirect pathway activity, thereby decreasing motivation to take substances of abuse, such as cocaine[41]. A recent effort utilizing a highly-miniaturized 1,536-well assay based on the Quest Fluo-8 calcium detection dye in D₂ and D₃ β -arrestin overexpressing cells resulted in the identification of a novel D₂ DAR antagonist series with excellent D₂ versus D₁, D₃, D₄, and D₅ receptor selectivity that carries the potential for treatment of multiple neuropsychiatric and endocrine disorders. [42,43]

Sensitization of adenylyl cyclase signaling has been implicated in a variety of neuropsychiatric and neurologic disorders including substance abuse and Parkinson's disease. Previous approaches to study sensitization have been generally cumbersome involving continuous cell culture maintenance as well as a complex methodology for measuring cAMP accumulation that involves multiple wash steps. Using two D₂ dopamine receptor cellular models, Conley et al. reported converting a laborious sensitization assay (>20 steps taking 4-5 days) to a five-step, single day assay in 384-well format, along with a demonstration that the assay design could also be readily used for reverse transfection of siRNA for future targeted siRNA library screening [44].

2.1.3. Acetylcholine—The muscarinic acetylcholine receptors (mAChRs) are members of the G Protein-Coupled Receptor (GPCR) family A that mediate the metabotropic actions of the neurotransmitter acetylcholine. To date, five distinct subtypes of mAChRs (M1-M5) have been cloned and sequenced, and blocking of M5 receptors has been shown to reduce reinforcement and withdrawal symptoms of morphine, as well as cocaine, addiction [45-47]. Using HTS in 1,536-well format, combined with medicinal chemistry efforts, Gentry *et al.* discovered ML381 (also referred to as VU0480131) as the most potent and selective M5-orthosteric antagonist reported to date[48]. Similarly, a functional high-throughput screen and subsequent medicinal chemistry effort identified the first mAChR negative allosteric modulator, ML375, with submicromolar potency and high selectivity for the M5 subtype. [49]

2.1.4. Glutamate—Though certain types of behavioral therapy have proven effective for treatment of cocaine addiction, relapse remains high, and there are currently no approved medications for the treatment of cocaine abuse. Recent evidence suggests a critical role for the metabotropic glutamate receptor subtype 5 (mGlu5) in the modulation of neural circuitry associated with the addictive properties of cocaine [50]. Using a 384-well based calcium mobilization assay, Amato *et al.* developed a potent and selective small molecule (VU0463841) with good CNS exposure in rats. Its utility was further demonstrated by its ability to attenuate drug seeking behaviors in relevant rat models of cocaine addiction[51].

Vesicular Monoamine Transporter 2 (VMAT2) inhibitors are also of interest for treatment of psychostimulant abuse and addiction. The natural product lobeline and its derivatives inhibit methamphetamine-induced dopamine release, as well as methamphetamine self-administration, *via* the inhibition of VMAT2. These compounds, structurally distinct from reserpine and tetrabenazine, are pursued as novel therapeutics in preclinical and clinical studies of methamphetamine abuse disorders. Recently, a new fluorescent probe, FFN206, was reported as an excellent VMAT2 substrate capable of detecting VMAT2 activity in intact cells using fluorescence microscopy. The probe was used in a cell-based fluorescence

assay using VMAT2-transfected HEK cells, with excellent Z' -factors of 0.7 – 0.8 reported[52].

2.1.5. Neurotensin (NT) receptors—Methamphetamine addiction remains a substantial public health issue and currently no small molecule therapies are available for its treatment. Neurotensin receptors are expressed on dopaminergic neurological pathways associated with reward and the neurotensin receptor 1 (NTR1) has been proposed as a therapeutic target for the treatment of methamphetamine abuse. NTR1 peptide agonists produce behaviors that are exactly opposite of the psychostimulant effects observed with methamphetamine abuse, such as hyperactivity, neurotoxicity, psychotic episodes, and cognitive deficits, and repeated administrations of NTR1 agonists do not lead to the development of tolerance. [53] A high-throughput screening campaign utilizing a 1,536-well-based calcium flux assay, followed by medicinal chemistry optimization, led to the discovery of ML314, a nonpeptidic β -arrestin biased agonist for NTR1[54].

2.1.6. G-protein coupled receptors and associated targets—The body of G-protein-activated inwardly rectifying potassium channel (GIRK) research implicates GIRK in diverse processes such as heart rhythm control, effects on reward/addiction, and modulation of response to analgesic [55]. GIRK regulation by GPCRs is believed to be linked to the biological effects of a variety of GPCR agonists, including opioids, acetylcholine, and the gamma-aminobutyric acid (GABA_B) receptor agonist baclofen. Representing a significant advancement in our ability to selectively probe GIRK's role in physiology, Kaufmann *et al.* recently reported the development and characterization of ML297 (VU0456810) as the first potent and selective GIRK activator; the molecule was discovered through a thallium-flux based screen of the NIH Molecular Libraries collection [56],[57]. GPR55 is a class A GPCR with roles in pain, metabolic disorder, bone development, and cancer [33]. In another screen of the Molecular Libraries collection, utilizing fluorescence-based β -arrestin 384-well assay, novel GPR55 antagonist chemotypes with IC₅₀ values in the range of 0.16–2.72 μ M were identified, serving as a starting point for further development[58].

Regulator of G-protein signaling (RGS) proteins potently suppress GPCR signal transduction by accelerating GTP hydrolysis on G-protein α [59]. RGS4 is overexpressed in the CNS and is proposed as a therapeutic target for treatment of neuropathological states including epilepsy and Parkinson's disease. An HEK293-FlpIn cell line stably expressing M3-muscarinic receptor with doxycycline-regulated RGS4 expression was employed to identify compounds that inhibit RGS4-mediated suppression of M3-muscarinic receptor signaling. Using this 384-well calcium-mediated fluorescence assay, several cell-active RGS4 inhibitors have been identified for use in future biological studies. [60]

2.1.7. cAMP levels—Both tolerance and dependence occur because frequent drug use can suppress components of the brain's reward circuit. The cAMP response element-binding protein (CREB) is a transcription factor with multiple targets within the cell. When drugs of abuse are administered, dopamine concentrations rise, leading to increased production of cAMP, which in turn activates CREB, leading to a multitude of downstream signaling events in part responsible for the development of addiction[22]. To develop tools aimed at

further understanding the biology surrounding cAMP/CREB and morphine addiction, the late Marshall Nirenberg used a cellular model system of neuroblastoma-glioma hybrid cells (NG108-15). To identify small molecules that can inhibit morphine-induced cAMP overshoot, a homogeneous time-resolved fluorescence (HTRF) cell-based assay that measures cellular cAMP production after morphine withdrawal was utilized with the above cell line. [61]

2.1.8. Neuropeptides—The role of neuropeptide Y Y2 receptor (Y2R) has been implicated in human diseases such as severe obesity, mood disorders, and alcoholism, but the currently available Y2R antagonists do not permit detailed biological studies to fully delineate the receptor's role. A high-throughput screening approach utilizing the FLIPR technology in 1,536-well format resulted in the identification of 5 selective, brain-penetrant small-molecule Y2R antagonists, with reportedly different mechanism of action from previously-reported agents. [62]

The neuropeptide galanin and the galanin receptor 3 (GalR3) have been implicated in addiction, specifically alcoholism, and mood-related disorders such as anxiety and depression[63]. Recently, the generation of a modified GalR3 that facilitates its cell surface expression, while maintaining wild-type receptor pharmacology, has been reported; it is expected that this platform, utilizing the FLIPR technology in 384-well format, will enable identification of GalR3-selective modulators to facilitate dissection of the biological role(s) that GalR3 plays in normal physiology and dysregulated states [64,65]

Neuropeptide S Receptor (NPSR) is a GPCR first described by Sato *et al.* in 2004 [66]. Utilizing an HTRF cAMP assay in 1,536-well format in combination with dose-response type screening, ML154, a structurally novel potent small molecule modulator of NPS-NPSR neurocircuitry, was discovered. ML154 is reportedly the most potent *in vivo* active NPSR-targeting compound to date and has superior microsomal stability compared to other lead NPSR antagonists disclosed in the literature, making it a good tool to use in studying NPS/NPSR pharmacology and the role of NPSR antagonism in sleep, anxiety, food intake, and addiction [17,67,68].

Orexin A and B, also known as hypocretin 1 and 2, are hypothalamic neuropeptides discovered in the late-1990s. They are the endogenous ligands for two GPCRs, orexin 1 (OX1) and orexin 2 (OX2). Increasing evidence implicates the OX1 receptor in reward processes, suggesting that OX1 antagonism could be used as a therapeutic intervention in drug addiction. Starting from an HTS using calcium fluorescence-based assay, and further optimizing hit series through medicinal chemistry, Perrey *et al.* reported the development of OX1-selective antagonists based on a tetrahydroisoquinoline scaffold[69].

2.2. Biochemical HTS Assays

While less well represented in the area of drug abuse research, biochemical assay platforms play a very important role in discovery of drug leads, particularly given the fact that they often allow the unambiguous identification of modulators of a discrete protein target; furthermore, biochemical assays often tend to be cheaper to implement on a large scale and may permit the evaluation of binding affinity.

Aldehyde dehydrogenase 2 (ALDH2) is an enzyme responsible for degradation of the metabolite acetaldehyde, the chemical responsible for triggering the red face in individuals sensitive to alcohol; modulation of acetaldehyde levels has also been exploited in the past through the use of disulfiram as an alcohol-abuse deterrent. Thus, highly-specific inhibition of ALDH2 may have a potential application for alcohol aversion therapy, and more recently, in cocaine addiction. Past efforts to discover ALDH2-selective inhibitors include *in vitro* enzyme activity screens as well as virtual computational screens using the structures of the target enzyme [70][71]. Recently, we developed highly-miniaturized 1,536-well assays to discover inhibitors of the broad family of dehydrogenase enzymes, including aldehyde dehydrogenases: the assay is based on the fluorescent detection of the NADH product resulting from the dehydrogenase-catalyzed reduction of its NAD⁺ cofactor, with detection performed in real-time kinetic format. Application of this assay in large-scale robotic screening has resulted in identification of multiple inhibitory chemotypes whose detailed characterization is ongoing[72].

RGS proteins, introduced earlier, are critical modulators of GPCR signaling given their ability to deactivate G_α subunits via GTPase-accelerating protein (GAP) activity. Several biochemical approaches to RGS targeting have been described recently. A Transcreeper® FP immunoassay based on a monoclonal antibody that recognizes GDP with greater than 100-fold selectivity over GTP has been used to detect the GTPase activity of a G_{αi1} mutant interacting with RGS4. [73,74] The assay, initially developed in 384-well format, has recently been further miniaturized to 1,536-well density and used to screen a ~400,000-compound collection in dose-response format[75]. Furthermore, RGS4 was highlighted in a Malachite Green assay for phosphate release: in a pilot screen using this assay, 13 compounds were identified and subjected to further analysis[76].

The transcriptional regulator FosB accumulates in the striatum in response to chronic administration of drugs of abuse, L-DOPA, or stress, with implications to the neurological changes associated with aspects of drug addiction. To discover chemical probes to study FosB, a high-throughput assay was developed to monitor the interaction of FosB with its target DNA sequence through the use of FP detection. From the resulting screen, two compounds capable of disrupting the interaction with low micromolar activity were identified. The two compounds displayed different activities against FosB homodimers compared to FosB/JunD heterodimers, suggesting that they could be useful in studies of the contribution of different FosB complexes to transcription regulation and to further evaluate FosB as a therapeutic target [77,78].

2.3. Model organism and related systems

The difficulty in translating fundamental biological discoveries into tangible therapies has further highlighted the need for better predictive technologies employed in early discovery. The need for better predictive models is applicable to the area of substance abuse research, as well [32]. Among lower model organisms, zebrafish (*Danio rerio*) have been the subject of growing interest, with recent reviews addressing their applicability as a model in general, and in particular applicability to substance abuse research [79-84][85-88]. While not immediately relevant to drug abuse screening, recent advances in assay development

utilizing zebrafish include target-based fluorescent reporter systems to track the level and distribution of proteins of interest [89], imaging algorithms to detect and quantitate blood vessel formation in zebrafish [90], and use of GFP-expressing transgenes to monitor differentiation into specific lineages [91].

Another lower organism that can be raised cost-effectively to be applied in HTS settings is the vinegar fly *Drosophila melanogaster*. Flies and vertebrates share many metabolic functions, molecular machinery, and analogous organ systems that control nutrient uptake, storage, and metabolism. In a recent screen for *Drosophila* larva feeding, a serotonin antagonist, metitepine, was identified as a potential modulator of feeding, with possible implications to eating disorders research[92]. The recently reported designer receptors exclusively activated by designer drugs (DREADDs), based on muscarinic acetylcholine GPCR, are also expected to serve as important tools to build *Drosophila* models of drug abuse [93]. In addition to zebrafish and the vinegar fly, *C. elegans* (*Caenorhabditis elegans*) researchers have made enormous strides to make this model worm HTS-amenable [94]. As methods are sought to predict a compound's efficacy and toxicity in man, having a battery of lower organism models employed in prioritization ahead of the more costly and lower-throughput rodent models will speed up the advancement of potential therapeutics.

Use of higher-level species, such as rodents, in drug abuse early discovery has been limited by cost considerations and, just as importantly, by doubts about the relevance of behavioral models based on rodents to the corresponding human physiology. Nonetheless, recent findings suggest that some behavioral abnormalities elicited by psychedelics in rodents might predict such effects in humans[95]. Further, technological advances in computer vision and machine learning are making it possible to conduct automated, potentially scaled-up, observational experiments to monitor complex animal behavior without the need for laborious human intervention: examples include the SmartCube[96], Pattern Array [97], PhenoMaster/LabMaster [98], INTELLICAGE systems [99], and the Behavioral Spectrometer[100].

3. Additional approaches

3.1. Active and passive immunization

As an alternative approach potentially superior to reacting, researchers have moved toward identifying patients with genetic traits that lead to predisposition to addiction and in turn have directed efforts toward finding ways to prevent such an addiction or its long-term effects. Anti-addiction vaccines, aimed at eliciting antibodies that block the pharmacological effects of drugs leading to addiction, have great potential for treating drug abuse [101-104]. Nicotine vaccines [105], in addition to those for cocaine and methamphetamine [106-108], are currently in various stages of development.

In addition to active immunization strategies, a properly designed passive immunization, also referred to as antidote or anti-serum, carries the potential to serve as a first-line defense in cases of severe drug overdose. An example of this line of research is the work by Janda and Koob to develop catalytic antibody-based treatment for cocaine overdose: a catalytic antibody could in principle dramatically accelerate the estherolytic conversion of cocaine to

an inactive derivative and thus help lower the cocaine blood levels in an overdose victim in emergency settings. Earlier research provided proof of concept by showing that the catalytic anti-cocaine monoclonal antibody GNC92H2, derived through an immunization with a cocaine-hydrolysis transition-state hapten and subsequent hybridoma production and clonal isolation, catalyzed cocaine hydrolysis and blocked the psychostimulatory effects of cocaine in animal model[109,110]. However, the reaction acceleration afforded by this antibody proved insufficient for its use in the clinic, necessitating the development of better catalysts. Nevertheless, a recent report has highlighted the potential to use a related phage-display strategy to efficiently deliver to the CNS, while protecting from degradation, either improved catalytic antibodies or enzymes such as butyrylcholinesterase, the major human cocaine-metabolizing enzyme, or bacterial cocaine esterase, the most efficient cocaine degradation enzyme, with the goal of achieving enhanced hydrolytic action on drugs of abuse[111].

3.2. High-throughput discovery of advantageous drug-drug combinations

Combination therapies offer the advantage of an increased positive effect, potentially at lower drug doses, and with associated lower toxicity. While the concept of using drug cocktails is not new, only very recently technological advances have allowed combination screening and data analysis to be performed on a truly large scale HTS [112]: in this study, the authors used acoustic dispense technology, coupled with improved informatics analysis processes, to develop a platform for the high-speed testing of many thousands of drug-drug combinations in a 6×6 concentration matrix, followed by a confirmatory testing of select pairs in a more detailed 10×10 matrix. It can be expected that the public availability of the protocols and software code for this matrix-type screening will spur efforts to re-screen established assays, potentially finding new therapeutic avenues.

In summary, early discovery efforts targeting drug abuse comprise a broad spectrum of platform, including traditional cell-based and biochemical assays, along with emerging technologies such as model-organism screening, automated animal-behavior monitoring, and high-throughput discovery of synergistic drug-drug combinations. Appreciation of the role of epigenetics is growing, as well.

4. Expert Opinion

Technologically, drug abuse drug discovery does not appear to be very different from the approaches employed in other disease types; recent examples from the literature provided within indicate that, with few exceptions, the assay formats and detection platforms used in drug abuse screening are the same as those employed elsewhere. The important difference lies in the enormous biological complexity associated with the control of processes such as mood, sense of satisfaction and reward, cravings, and others, that collectively conspire to drive an individual down the path of addiction. With such complexity comes the associated difficulty in finding “good” targets and achieving exquisite selectivity of potential therapeutic agents. As an added demand, sometimes drugs against drug abuse need to be extremely safe and devoid of side effects as they are likely to be applied for extended periods of the subject's life. On the other hand, there exist possibilities for short term

treatments to help break addictions whereby a drug against drug abuse would not be administered over long periods.

Complicating factors such as those described above make it especially important for the field to accelerate utilization of next-generation methods and models, and to seek novel therapeutic targets. To this end, efforts have been growing the areas of drug repurposing [113], as well as to gain a better understanding of the role of epigenetic regulation in the predisposition to and genesis of addiction [114-116], with a growing interest in the role of microbiome also being anticipated. Along with the already-reviewed platforms to discover novel drug-drug combinations and increased use of automated whole-animal behavioral screening systems, the employment of organoid-level three-dimensional test platforms (also referred to as tissue-chip or organs-on-chip), which carry the promise of recapitulating the complexity inherent to whole-organism physiology, will be a crucial next step towards better validating initial discoveries made using the more reductionist traditional cell-culture type screens.

While the negative effects of drugs of abuse have been described in detail, it is only relatively recently that potential benefits of these drugs, for which there exist vast clinical, efficacy, and toxicity data from human use, have begun to be investigated. With repurposing now at the forefront of drug discovery programs, public-access programs such as the Molecular Pharmacology Research Program [117] and the NIMH Psychoactive Drug Screening Program [118,119] are allowing for these bioavailable and efficacious compounds to be tested in order to determine what other diseases they may have the potential to treat. Recent examples of the novel use of the psychedelic psilocybin [120,121] and ecstasy [122] for depression and PTSD, respectively, show the potential of repurposing of compounds with known clinical efficacy, and argues for including them in screening collections [123].

In closing, it is worth pointing out again that substance/drug abuse represents an extreme form of “evolution”: while the above examples show how the leading substances currently being abused are being targeted (through therapeutic interventions and - though not the subject of this review – through law enforcement, regulatory, and other mechanisms), there is a just-as-strong of a trend within the drug abuse community to seek new substances that would be undetectable or are currently unregulated so that the addiction, and associated profits thereof, can continue unabated. Indeed, recent publications highlight the rising tide of synthetic substances that sometimes represent constantly-changing chemical analogues of an initial addictive molecule which traditional management approaches through testing and legislative measures fail to capture rapidly enough [124-128].

References

1. www.Drugabuse.Gov/publications/media-guide/science-drug-abuse-addiction.
2. www.Drugabuse.Gov/publications/addiction-science-molecules-to-managed-care/introduction/drug-abuse-costs-united-states-economy-hundreds-billions-dollars-in-increased-health.
3. www.Drugabuse.Gov/drugs-abuse/commonly-abused-drugs/commonly-abused-drugs-chart.
4. Fulton BS. Medication development for the treatment of substance abuse. Annual reports in medicinal chemistry, vol 43. 2008; 43:61–72.

5. Kenna GA, Nielsen DM, Mello P, Schiesl A, Swift RM. Pharmacotherapy of dual substance abuse and dependence. *Cns Drugs*. 2007; 21(3):213–237. [PubMed: 17338593]
- 6*. Julien, RM. A primer of drug action : A concise, nontechnical guide to the actions, uses, and side effects of psychoactive drugs. Worth Publishers; New York: 2001. Textbook covers and communicates clearly the “actions, uses, and side effects of psychoactive drugs” An outstanding source for beginners in the substance abuse field
7. Harwood, HJ.; Myers, TG. New treatments for addiction: Behavioral, ethical, legal, and social questions. National Academies Press; 2004.
8. Rapaka, RS.; Sadée, W. Drug addiction : From basic research to therapy. Springer, AAPS Press; New York, NY: 2008.
9. Charney, DS.; Nestler, EJ.; Sklar, P.; Buxbaum, JD. Neurobiology of mental illness. Oxford University Press; 2013.
- 10*. Acri, JB.; Skolnick, P. Pharmacotherapy of substance use disorders. 4th ed. In: Charney EJM, Dennis S.; Sklar, Pamela; Buxbaum, Joseph D., editors. Neurobiology of mental illness. Oxford University Press; London: 2013. Comprehensive overview on pharmacotherapies (small molecule, biologicals, vaccines) available for substance abuse
11. Suvarna V. Phase iv of drug development. *Perspect Clin Res*. 2010; 1(2):57–60. [PubMed: 21829783]
12. www.Forbes.Com/sites/davidkroll/2013/05/11/why-does-arenas-obesity-drug-have-abuse-potential/.
13. Shram MJ, Schoedel KA, Bartlett C, Shazer RL, Anderson CM, Sellers EM. Evaluation of the abuse potential of lorcaserin, a serotonin 2c (5-HT_{2C}) receptor agonist, in recreational polydrug users. *Clinical pharmacology and therapeutics*. 2011; 89(5):683–692. [PubMed: 21412231]
14. Heger M. State challenges to painkiller could threaten fda authority. *Nat Med*. 2014; 20(5):453–453. [PubMed: 24804739]
15. www.thefix.com/content/inside-addiction-drug-pipeline-vaccines-Scripps8242?page=all.
- 16*. Macarron R, Banks MN, Bojanic D, Burns DJ, Cirovic DA, Garyantes T, Green DV, Hertzberg RP, Janzen WP, Paslay JW, Schopfer U, et al. Impact of high-throughput screening in biomedical research. *Nat Rev Drug Discov*. 2011; 10(3):188–195. Provides examples of approved drugs derived through high-throughput screening and the HTS impact on the drug discovery process. [PubMed: 21358738]
- 17*. Forray A, Sofuoglu M. Future pharmacological treatments for substance use disorders. *Brit J Clin Pharmacol*. 2014; 77(2):382–400. Detailed review of compounds that are used for substance abuse, along with their target, MOA, and efficacy information.
18. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F. Addiction: Beyond dopamine reward circuitry. *Proc Natl Acad Sci U S A*. 2011; 108(37):15037–15042. [PubMed: 21402948]
- 19*. Volkow ND, Skolnick P. New medications for substance use disorders: Challenges and opportunities. *Neuropsychopharmacology*. 2012; 37(1):290–292. Excellent review on the current therapies for substance abuse, with a detailed list of targets and their effects in animal models. [PubMed: 22157859]
20. Ruiz, P.; Strain, EC.; Langrod, J. The substance abuse handbook. Lippincott Williams & Wilkins; 2007.
21. Lowinson, JH. Substance abuse: A comprehensive textbook. Lippincott Williams & Wilkins; 2005.
22. Nestler EJ, Malenka RC. The addicted brain. *Scientific American*. 2004; 290(3):78–85. [PubMed: 14981881]
23. www.Drugabuse.Gov/publications/drugfacts/treatment-approaches-drug-addiction.
24. Volkow ND, Baler RD. Addiction science: Uncovering neurobiological complexity. *Neuropharmacology*. 2014; 76(B):235–249. [PubMed: 23688927]
25. Inglese J, Auld DS, Jadhav A, Johnson RL, Simeonov A, Yasgar A, Zheng W, Austin CP. Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc Natl Acad Sci U S A*. 2006; 103(31):11473–11478. [PubMed: 16864780]
26. Montoya ID. Advances in the development of biologics to treat drug addictions and overdose. *Adicciones*. 2012; 24(2):95–103. [PubMed: 22648312]

27. Kinsey B. Vaccines against drugs of abuse: Where are we now? *Therapeutic Advances in Vaccines*. 2014; 2(4):106–117. [PubMed: 24982760]
28. Michael S, Auld D, Klumpp C, Jadhav A, Zheng W, Thorne N, Austin CP, Inglese J, Simeonov A. A robotic platform for quantitative high-throughput screening. *Assay Drug Dev Technol*. 2008; 6(5):637–657. [PubMed: 19035846]
29. Seethala, R.; Zhang, L. *Handbook of drug screening*. Informa Healthcare; New York: 2009.
30. Yasgar A, Shinn P, Jadhav A, Auld D, Michael S, Zheng W, Austin CP, Inglese J, Simeonov A. Compound management for quantitative high-throughput screening. *JALA Charlottesville Va*. 2008; 13(2):79–89. [PubMed: 18496600]
31. Jin G, Wong ST. Toward better drug repositioning: Prioritizing and integrating existing methods into efficient pipelines. *Drug Discov Today*. 2014; 19(5):637–644. [PubMed: 24239728]
- 32**. Enna SJ, Williams M. Challenges in the search for drugs to treat central nervous system disorders. *J Pharmacol Exp Ther*. 2009; 329(2):404–411. Thorough review on CNS disorders, covering the drug discovery process, providing positive and negative examples for target vs. phenotypic methodologies for CNS disorders, and examples of automated screens for rodent behavior. [PubMed: 19182069]
- 33*. Xie XQ, Wang L, Liu H, Ouyang Q, Fang C, Su W. Chemogenomics knowledgebasedpolypharmacology analyses of drug abuse related g-protein coupled receptors and their ligands. *Front Pharmacol*. 2014; 5(3):1–11. A comprehensive and recent review featuring a list of GPCRs implicated in substance abuse. Provides detailed information on each receptor. [PubMed: 24478702]
34. Law, PY. *The opiate receptors*. Springer; 2011. Opioid receptor signal transduction mechanisms; p. 195–238.
35. Bart G. Maintenance medication for opiate addiction: The foundation of recovery. *J Addict Dis*. 2012; 31(3):207–225. [PubMed: 22873183]
36. White KL, Scopton AP, Rives ML, Bikbulatov RV, Polepally PR, Brown PJ, Kenakin T, Javitch JA, Zjawiony JK, Roth BL. Identification of novel functionally selective kappa-opioid receptor scaffolds. *Mol Pharmacol*. 2014; 85(1):83–90. [PubMed: 24113749]
37. Zhou L, Lovell KM, Frankowski KJ, Slauson SR, Phillips AM, Streicher JM, Stahl E, Schmid CL, Hodder P, Madoux F, Cameron MD, et al. Development of functionally selective, small molecule agonists at kappa opioid receptors. *J Biol Chem*. 2013; 288(51):36703–36716. [PubMed: 24187130]
38. Higgins GA, Sellers EM, Fletcher PJ. From obesity to substance abuse: Therapeutic opportunities for 5-HT_{2C} receptor agonists. *Trends Pharmacol Sci*. 2013; 34(10):560–570. [PubMed: 24041919]
39. Cornelius P, Lee E, Lin W, Wang R, Werner W, Brown JA, Stuhmeier F, Boyd JG, McClure K. Design, synthesis, and pharmacology of fluorescently labeled analogs of serotonin: Application to screening of the 5-HT_{2C} receptor. *J Biomol Screen*. 2009; 14(4):360–370. [PubMed: 19403919]
40. Jorgensen S, Nielsen EO, Peters D, Dyhring T. Validation of a fluorescence-based high-throughput assay for the measurement of neurotransmitter transporter uptake activity. *J Neurosci Methods*. 2008; 169(1):168–176. [PubMed: 18222006]
41. Picciotto MR. An indirect resilience to addiction. *Nat Neurosci*. 2013; 16(5):521–523. [PubMed: 23619754]
42. Xiao, J.; Free, RB.; Barnaeva, E.; Conroy, J.; Doyle, T.; Bryant-Genevier, M.; Taylor, MK.; Southall, N.; Hu, X.; Ferrer, M.; Titus, S., et al. Probe reports from the nih molecular libraries program. Bethesda (MD): 2010. Discovery, optimization, and characterization of a novel series of dopamine d₂ versus d₃ receptor selective antagonists.
43. Xiao J, Free RB, Barnaeva E, Conroy JL, Doyle T, Miller B, Bryant-Genevier M, Taylor MK, Hu X, Dulcey AE, Southall N, et al. Discovery, optimization, and characterization of novel d₂ dopamine receptor selective antagonists. *J Med Chem*. 2014; 57(8):3450–3463. [PubMed: 24666157]
44. Conley JM, Brust TF, Xu R, Burris KD, Watts VJ. Drug-induced sensitization of adenylyl cyclase: Assay streamlining and miniaturization for small molecule and siRNA screening applications. *J Vis Exp*. 2014; 83 e51218.

45. Weaver CD, Sheffler DJ, Lewis LM, Bridges TM, Williams R, Nalywajko NT, Kennedy JP, Mulder MM, Jadhav S, Aldrich LA, Jones CK, et al. Discovery and development of a potent and highly selective small molecule muscarinic acetylcholine receptor subtype i (machr 1 or m1) antagonist in vitro and in vivo probe. *Curr Top Med Chem*. 2009; 9(13):1217–1226. [PubMed: 19807667]
46. Jakubik J, Santruckova E, Randakova A, Janickova H, Zimcik P, Rudajev V, Michal P, El-Fakahany EE, Dolezal V. Outline of therapeutic interventions with muscarinic receptor-mediated transmission. *Physiol Res*. 2014; 63 Suppl 1:S177–189. [PubMed: 24564657]
47. Raffa RB. The m-5 muscarinic receptor as possible target for treatment of drug abuse. *J Clin Pharm Ther*. 2009; 34(6):623–629. [PubMed: 20175795]
48. Gentry PR, Kokubo M, Bridges TM, Cho HP, Smith E, Chase P, Hodder PS, Utlej TJ, Rajapakse A, Byers F, Niswender CM, et al. Discovery, synthesis and characterization of a highly muscarinic acetylcholine receptor (machr)-selective m -orthosteric antagonist, vu0488130 (ml381): A novel molecular probe. *ChemMedChem*. 2014
49. Gentry PR, Kokubo M, Bridges TM, Kett NR, Harp JM, Cho HP, Smith E, Chase P, Hodder PS, Niswender CM, Daniels JS, et al. Discovery of the first m5-selective and cns penetrant negative allosteric modulator (nam) of a muscarinic acetylcholine receptor: (s)-9b-(4-chlorophenyl)-1-(3,4-difluorobenzoyl)-2,3-dihydro-1h-imidazo[2,1-a]isoindol-5(9bh)-one (ml375). *J Med Chem*. 2013; 56(22):9351–9355. [PubMed: 24164599]
50. Emmitte KA. Recent advances in the design and development of novel negative allosteric modulators of mglu(5). *ACS Chem Neurosci*. 2011; 2(8):411–432. [PubMed: 21927649]
51. Amato RJ, Felts AS, Rodriguez AL, Venable DF, Morrison RD, Byers FW, Daniels JS, Niswender CM, Conn PJ, Lindsley CW, Jones CK, et al. Substituted 1-phenyl-3-(pyridin-2-yl)urea negative allosteric modulators of mglu5: Discovery of a new tool compound vu0463841 with activity in rat models of cocaine addiction. *ACS Chem Neurosci*. 2013; 4(8):1217–1228. [PubMed: 23682684]
52. Hu G, Henke A, Karpowicz RJ Jr, Sonders MS, Farrimond F, Edwards R, Sulzer D, Sames D. New fluorescent substrate enables quantitative and high-throughput examination of vesicular monoamine transporter 2 (vmat2). *ACS Chem Biol*. 2013; 8(9):1947–1954. [PubMed: 23859623]
53. Hershberger PM, Hedrick MP, Peddibhotla S, Mangravita-Novo A, Gosalia P, Li Y, Gray W, Vicchiarelli M, Smith LH, Chung TD, Thomas JB, et al. Imidazole-derived agonists for the neurotensin 1 receptor. *Bioorg Med Chem Lett*. 2014; 24(1):262–267. [PubMed: 24332089]
54. Peddibhotla S, Hedrick MP, Hershberger P, Maloney PR, Li Y, Milewski M, Gosalia P, Gray W, Mehta A, Sugarman E, Hood B, et al. Discovery of ml314, a brain penetrant non-peptidic beta-arrestin biased agonist of the neurotensin ntr1 receptor. *ACS Med Chem Lett*. 2013; 4(9):846–851. [PubMed: 24611085]
55. Nagisa S, Toru K, Kazutaka I. Role of girk channels in addictive substance effects. *Journal of Drug and Alcohol Research*. 2013; 2(2013):1–11.
56. Lujan R, de Velasco EMF, Aguado C, Wickman K. New insights into the therapeutic potential of girk channels. *Trends Neurosci*. 2014; 37(1):20–38. [PubMed: 24268819]
57. Kaufmann K, Romaine I, Days E, Pascual C, Malik A, Yang L, Zou B, Du Y, Sliwoski G, Morrison RD, Denton J, et al. MI297 (vu0456810), the first potent and selective activator of the girk potassium channel, displays antiepileptic properties in mice. *ACS Chem Neurosci*. 2013; 4(9):1278–1286. [PubMed: 23730969]
58. Kotsikourou E, Sharir H, Shore DM, Hurst DP, Lynch DL, Madrigal KE, Heynen-Genel S, Milan LB, Chung TD, Seltzman HH, Bai Y, et al. Identification of the gpr55 antagonist binding site using a novel set of high-potency gpr55 selective ligands. *Biochemistry*. 2013; 52(52):9456–9469. [PubMed: 24274581]
59. Roman DL, Traynor JR. Regulators of g protein signaling (rgs) proteins as drug targets: Modulating g-protein-coupled receptor (gpcr) signal transduction miniperspective. *J Med Chem*. 2011; 54(21):7433–7440. [PubMed: 21916427]
60. Storaska AJ, Mei JP, Wu M, Li M, Wade SM, Blazer LL, Sjogren B, Hopkins CR, Lindsley CW, Lin Z, Babcock JJ, et al. Reversible inhibitors of regulators of g-protein signaling identified in a high-throughput cell-based calcium signaling assay. *Cell Signal*. 2013; 25(12):2848–2855. [PubMed: 24041654]

61. Xia M, Guo V, Huang R, Shahane SA, Austin CP, Nirenberg M, Sharma SK. Inhibition of morphine-induced camp overshoot: A cell-based assay model in a high-throughput format. *Cell Mol Neurobiol.* 2011; 31(6):901–907. [PubMed: 21598037]
62. Brothers SP, Saldanha SA, Spicer TP, Cameron M, Mercer BA, Chase P, McDonald P, Wahlestedt C, Hodder PS. Selective and brain penetrant neuropeptide y y2 receptor antagonists discovered by whole-cell high-throughput screening. *Mol Pharmacol.* 2010; 77(1):46–57. [PubMed: 19837904]
63. Belfer I, Hipp H, Bollettino A, McKnight C, Evans C, Virkkunen M, Albaugh B, Max MB, Goldman D, Enoch MA. Alcoholism is associated with galr3 but not two other galanin receptor genes. *Genes Brain Behav.* 2007; 6(5):473–481. [PubMed: 17083333]
64. Robinson J, Smith A, Sturchler E, Tabrizifard S, Kamenecka T, McDonald P. Development of a high-throughput screening-compatible cell-based functional assay to identify small molecule probes of the galanin 3 receptor (galr3). *Assay Drug Dev Technol.* 2013; 11(8):468–477. [PubMed: 24116939]
65. Holmes A, Picciotto MR. Galanin: A novel therapeutic target for depression, anxiety disorders and drug addiction? *CNS Neurol Disord Drug Targets.* 2006; 5(2):225–232. [PubMed: 16611095]
66. Cannella N, Kallupi M, Ruggeri B, Ciccocioppo R, Ubaldi M. The role of the neuropeptide s system in addiction: Focus on its interaction with the crf and hypocretin/orexin neurotransmission. *Prog Neurobiol.* 2013; 100(January 2013):48–59. [PubMed: 23041581]
67. Marugan, J.; Liu, K.; Zheng, W.; Eskay, R.; Southall, N.; Heilig, M.; Inglese, J.; Austin, C. Probe reports from the nih molecular libraries program. Bethesda (MD): 2010. Identification of functionally selective small molecule antagonists of the neuropeptide-s receptor: Naphthopyranopyrimidines.
68. Ashare RL, Schmidt HD. Optimizing treatments for nicotine dependence by increasing cognitive performance during withdrawal. *Expert Opin Drug Discov.* 2014; 9(6):579–594. [PubMed: 24707983]
69. Perrey DA, German NA, Gilmour BP, Li JX, Harris DL, Thomas BF, Zhang YA. Substituted tetrahydroisoquinolines as selective antagonists for the orexin 1 receptor. *J Med Chem.* 2013; 56(17):6901–6916. [PubMed: 23941044]
70. Parajuli B, Kimble-Hill AC, Khanna M, Ivanova Y, Meroueh S, Hurley TD. Discovery of novel regulators of aldehyde dehydrogenase isoenzymes. *Chem Biol Interact.* 2011; 191(1-3):153–158. [PubMed: 21349255]
71. Koppaka V, Thompson DC, Chen Y, Ellermann M, Nicolaou KC, Juvonen RO, Petersen D, Deitrich RA, Hurley TD, Vasiliou V. Aldehyde dehydrogenase inhibitors: A comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. *Pharmacol Rev.* 2012; 64(3):520–539. [PubMed: 22544865]
72. [pubchem.Ncbi.Nlm.Nih.Gov/assay/assay.cgi?Aid=1030](https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?Aid=1030).
73. Zielinski T, Kimple AJ, Hutsell SQ, Koeff MD, Siderovski DP, Lowery RG. Two galpha(i1) rate-modifying mutations act in concert to allow receptor-independent, steady-state measurements of rgs protein activity. *J Biomol Screen.* 2009; 14(10):1195–1206. [PubMed: 19820068]
74. Traynor J. Regulator of g protein-signaling proteins and addictive drugs. *Ann N Y Acad Sci.* 2010; 1187(1):341–352. [PubMed: 20201861]
75. [pubchem.Ncbi.Nlm.Nih.Gov/assay/assay.cgi?Aid=504845](https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?Aid=504845).
76. Monroy CA, Mackie DI, Roman DL. A high throughput screen for rgs proteins using steady state monitoring of free phosphate formation. *PLoS One.* 2013; 8(4)
77. Wang Y, Cesena TI, Ohnishi Y, Burger-Caplan R, Lam V, Kirchhoff PD, Larsen SD, Larsen MJ, Nestler EJ, Rudenko G. Small molecule screening identifies regulators of the transcription factor delta fosb. *ACS Chem Neurosci.* 2012; 3(7):546–556. [PubMed: 22860224]
78. Glass MJ. Opioid dependence and nmda receptors. *Ilar J.* 2011; 52:342–351. [PubMed: 23382148]
79. Collier AD, Khan KM, Caramillo EM, Mohn RS, Echevarria DJ. Zebrafish and conditioned place preference: A translational model of drug addiction. *Prog Neuropsychopharmacol Biol Psychiatry.* 2014
80. Stewart A, Wong K, Cachat J, Gaikwad S, Kyzar E, Wu N, Hart P, Piet V, Utterback E, Elegante M, Tien D, et al. Zebrafish models to study drug abuse-related phenotypes. *Rev Neurosci.* 2011; 22(1):95–105. [PubMed: 21615264]

81. Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol Sci.* 2014; 35(2):63–75. [PubMed: 24412421]
- 82*. Giacomotto J, Segalat L. High-throughput screening and small animal models, where are we? *Brit J Pharmacol.* 2010; 160(2):204–216. An excellent overview of HTS-amenable lower-organism models. [PubMed: 20423335]
83. Wielhouwer EM, Ali S, Al-Afandi A, Blom MT, Riekerink MBO, Poelma C, Westerweel J, Oonk J, Vrouwe EX, Buesink W, vanMil HGJ, et al. Zebrafish embryo development in a microfluidic flow-through system. *Lab Chip.* 2011; 11(10):1815–1824. [PubMed: 21491052]
84. Mathias JR, Saxena MT, Mumm JS. Advances in zebrafish chemical screening technologies. *Future Med Chem.* 2012; 4(14):1811–1822. [PubMed: 23043478]
85. Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff AV. Zebrafish models for translational neuroscience research: From tank to bedside. *Trends Neurosci.* 2014; 37(5):264–278. [PubMed: 24726051]
86. Cavodeassi F, Bene FD, Fürthauer M, Grabher C, Herzog W, Lehtonen S, Linker C, Mercader N, Mikut R, Norton W. Report of the second european zebrafish principal investigator meeting in karlsruhe, germany, march 21–24, 2012. *Zebrafish.* 2013; 10(1):119–123. [PubMed: 23530760]
87. Neelkantan N, Mikhaylova A, Stewart AM, Arnold R, Gjeloši V, Kondaveeti D, Poudel MK, Kalueff AV. Perspectives on zebrafish models of hallucinogenic drugs and related psychotropic compounds. *ACS Chem Neurosci.* 2013; 4(8):1137–1150. [PubMed: 23883191]
88. Cousin MA, Ebbert JO, Wiinamaki AR, Urban MD, Argue DP, Ekker SC, Klee EW. Larval zebrafish model for fda-approved drug repositioning for tobacco dependence treatment. *PLoS One.* 2014; 9(3):e90467. [PubMed: 24658307]
89. Yozzo KL, Isales GM, Raftery TD, Volz DC. High-content screening assay for identification of chemicals impacting cardiovascular function in zebrafish embryos. *Environ Sci Technol.* 2013; 47(19):11302–11310. [PubMed: 24015875]
90. Huang HG, Lindgren A, Wu XR, Liu NA, Lin SO. High-throughput screening for bioactive molecules using primary cell culture of transgenic zebrafish embryos. *Cell Rep.* 2012; 2(3):695–704. [PubMed: 22999940]
91. Tran TC, Sneed B, Haider J, Blavo D, White A, Aiyejorun T, Baranowski TC, Rubinstein AL, Doan TN, Dingledine R, Sandberg EM. Automated, quantitative screening assay for antiangiogenic compounds using transgenic zebrafish. *Cancer Res.* 2007; 67(23):11386–11392. [PubMed: 18056466]
92. Gasque G, Conway S, Huang J, Rao Y, Voshall LB. Small molecule drug screening in drosophila identifies the 5ht2a receptor as a feeding modulation target. *Scientific reports.* 2013; 3(2120):1–8.
93. Becnel J, Johnson O, Majeed ZR, Tran V, Yu B, Roth BL, Cooper RL, Kerut EK, Nichols CD. Dredds in drosophila: A pharmacogenetic approach for controlling behavior, neuronal signaling, and physiology in the fly. *Cell Rep.* 2013; 4(5):1049–1059. [PubMed: 24012754]
94. O'Reilly LP, Luke CJ, Perlmutter DH, Silverman GA, Pak SC. C. Elegans in high-throughput drug discovery. *Adv Drug Deliv Rev.* 2014; 2014(69-70):247–253. [PubMed: 24333896]
95. Hanks JB, Gonzalez-Maeso J. Animal models of serotonergic psychedelics. *ACS Chem Neurosci.* 2013; 4(1):33–42. [PubMed: 23336043]
96. Tecott LH, Nestler EJ. Neurobehavioral assessment in the information age. *Nat Neurosci.* 2004; 7(5):462–466. [PubMed: 15114359]
97. Kafafi N, Mayo CL, Elmer GI. Mining mouse behavior for patterns predicting psychiatric drug classification. *Psychopharmacology (Berl).* 2014; 231(1):231–242. [PubMed: 23958942]
98. Portal E, Riess O, Nguyen HP. Automated home cage assessment shows behavioral changes in a transgenic mouse model of spinocerebellar ataxia type 17. *Behav Brain Res.* 2013; 250:157–165. [PubMed: 23665119]
99. Lipp HP. High-throughput and automated behavioural screening of normal and genetically modified mice. *Bus Brief Future Drug Discov.* 2005:1–5.
100. Brodtkin J, Frank D, Grippo R, Hausfater M, Gulinello M, Achterholt N, Gutzen C. Validation and implementation of a novel high-throughput behavioral phenotyping instrument for mice. *J Neurosci Methods.* 2014; 224:48–57. [PubMed: 24384067]

101. Moreno AY, Janda KD. Current challenges for the creation of effective vaccines against drugs of abuse. *Expert Rev Vaccines*. 2011; 10(12):1637–1639. [PubMed: 22085165]
102. Shen XY, Kosten TR. Immunotherapy for drug abuse. *Cns Neurol Disord-Dr*. 2011; 10(8):876–879.
103. Chi KR. Vaccines move forward against a range of addictions. *Nat Med*. 2011; 17(2):146. [PubMed: 21297598]
104. Hipser C, Bushlin I, Gupta A, Gomes I, Devi LA. Role of antibodies in developing drugs that target g-protein-coupled receptor dimers. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*. 2010; 77(4):374–380.
105. Pentel P, Lesage M. New directions in nicotine vaccine design and use. *Advances in pharmacology (San Diego, Calif)*. 2013; 69:553–580.
106. Miller ML, Moreno AY, Aarde SM, Creehan KM, Vandewater SA, Vaillancourt BD, Wright MJ, Janda KD, Taffe MA. A methamphetamine vaccine attenuates methamphetamine-induced disruptions in thermoregulation and activity in rats. *Biol Psychiatry*. 2013; 73(8):721–728. [PubMed: 23098894]
- 107*. Brimijoin S, Shen XY, Orson F, Kosten T. Prospects, promise and problems on the road to effective vaccines and related therapies for substance abuse. *Expert Rev Vaccines*. 2013; 12(3): 323–332. Recent review highlighting the various vaccine methodologies available for substance, specifically examples with cocaine and nicotine. [PubMed: 23496671]
108. Shen XY, Orson FM, Kosten TR. Vaccines against drug abuse. *Clin Pharmacol Ther*. 2012; 91(1):60–70. [PubMed: 22130115]
109. Carrera MR, Ashley JA, Zhou B, Wirsching P, Koob GF, Janda KD. Cocaine vaccines: Antibody protection against relapse in a rat model. *Proc Natl Acad Sci U S A*. 2000; 97(11):6202–6206. [PubMed: 10823960]
110. Carrera MRA, Ashley JA, Wirsching P, Koob GF, Janda KD. A second-generation vaccine protects against the psychoactive effects of cocaine. *Proc Natl Acad Sci U S A*. 2001; 98(4): 1988–1992. [PubMed: 11172063]
111. Bazan J, Calkosinski I, Gamian A. Phage display--a powerful technique for immunotherapy: 2. Vaccine delivery *Hum Vaccin Immunother*. 2012; 8(12):1829–1835. [PubMed: 22906938]
112. Mathews Griner LA, Guha R, Shinn P, Young RM, Keller JM, Liu D, Goldlust IS, Yasgar A, McKnight C, Boxer MB, Duvéau DY, et al. High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated b-cell-like diffuse large b-cell lymphoma cells. *Proc Natl Acad Sci U S A*. 2014; 111(6):2349–2354. [PubMed: 24469833]
- 113*. Sekhon BS. Repositioning drugs and biologics: Retargeting old/existing drugs for potential new therapeutic applications. *Journal of Pharmaceutical Education & Research*. 2013; 4(1) Authors provide an extensive list of repositioned drugs.
114. Robison AJ, Nestler EJ. Transcriptional and epigenetic mechanisms of addiction. *Nat Rev Neurosci*. 2011; 12(11):623–637. [PubMed: 21989194]
- 115*. Nestler EJ. Epigenetic mechanisms of drug addiction. *Neuropharmacology*. 2014; 76 Pt B:259–268. Recent review covering all aspects (histone modification, DNA methylation, noncoding RNAs) of epigenetic regulation in relation to substance abuse. [PubMed: 23643695]
116. Tuesta LM, Zhang Y. Mechanisms of epigenetic memory and addiction. *Embo J*. 2014; 33(10): 1091–1103. [PubMed: 24778453]
117. www.Nimh.Nih.Gov/about/organization/dnbbs/molecular-cellular-and-genomic-neuroscience-research-branch/molecular-pharmacology-research-program.Shtml.
118. pdsp.Med.Unc.Edu/indexr.Html.
119. www.Nature.Com/news/2009/091101/full/news.2009.1047.Html.
120. Kupferschmidt K. High hopes. *Science*. 2014; 345(6192):18–23. [PubMed: 24994630]
121. Tagliazucchi E, Carhart-Harris R, Leech R, Nutt D, Chialvo DR. Enhanced repertoire of brain dynamical states during the psychedelic experience. *Human Brain Mapping*. 2014 n/a-n/a.
122. Kupferschmidt K. Can ecstasy treat the agony of ptsd? *Science*. 2014; 345(6192):22–23. [PubMed: 24994631]

123. Huang R, Southall N, Wang Y, Yasgar A, Shinn P, Jadhav A, Nguyen DT, Austin CP. The ncgc pharmaceutical collection: A comprehensive resource of clinically approved drugs enabling repurposing and chemical genomics. *Science translational medicine*. 2011; 3(80) 80ps16.
124. www.Unodc.Org/documents/scientific/nps_2013_smart.Pdf.
125. www.Economist.Com/news/international/21602729-traditional-drugs-lose-their-lure-novel-ones-are-filling-gap-market-new.
126. Westwell AD, Caldicott DG, Hutchings A. The dark side of pharmaceutical chemistry. *Future Med Chem*. 2012; 4(2):129–132. [PubMed: 22300092]
127. Nichols D. Legal highs: The dark side of medicinal chemistry. *Nature*. 2011; 469(7328):7. [PubMed: 21209630]
128. Appendino G, Minassi A, Taglialatela-Scafati O. Recreational drug discovery: Natural products as lead structures for the synthesis of smart drugs. *Natural product reports*. 2014
129. Gentry PR, Bridges TM, Lamsal A, Vinson PN, Smith E, Chase P, Hodder PS, Engers JL, Niswender CM, Daniels JS, Jeffrey Conn P, et al. Discovery of ml326: The first sub-micromolar, selective m5 pam. *Bioorg Med Chem Lett*. 2013; 23(10):2996–3000. [PubMed: 23562060]
130. Frankowski KJ, Hedrick MP, Gosalia P, Li K, Shi S, Whipple D, Ghosh P, Prisinzano TE, Schoenen FJ, Su Y, Vasile S, et al. Discovery of small molecule kappa opioid receptor agonist and antagonist chemotypes through a hts and hit refinement strategy. *ACS Chem Neurosci*. 2012; 3(3):221–236. [PubMed: 22737280]
131. Diaz JL, Christmann U, Fernandez A, Luengo M, Bordas M, Enrech R, Carro M, Pascual R, Burgueno J, Merlos M, Benet-Buchholz J, et al. Synthesis and biological evaluation of a new series of hexahydro-2h-pyrano[3,2-c]quinolines as novel selective sigma1 receptor ligands. *J Med Chem*. 2013; 56(9):3656–3665. [PubMed: 23560650]

List of abbreviations

IC₅₀	concentration that produces 50% inhibition
HTS	high-throughput screening
PubChem AID	PubChem Assay Identifier
MOA	mechanism of action
SAR	structure-activity relationship
HTRF	homogeneous time-resolved fluorescence
BRET	bioluminescence resonance energy transfer
cAMP	cyclic adenosine monophosphate
CREB	cAMP response element-binding protein
FP	fluorescence polarization
NIDA	The National Institute on Drug Abuse
KOR	κ-opioid receptor
DAT	dopamine
NET	norepinephrine
SERT	serotonin
AC	adenylyl cyclase
mAChRs	muscarinic acetylcholine receptors

GPCR	G Protein-Coupled Receptor
mGlu5	metabotropic glutamate receptor subtype 5
VMAT2	Vesicular Monoamine Transporter 2
NTR1	neurotensin receptor 1
OX1	orexin 1
OX2	orexin 2
ALDH2	aldehyde dehydrogenase 2
GAP	GTPase-accelerating protein

Highlights

- In addition to traditional target- and pathway-based cell-based high-throughput screening to discover modulators of addiction, biochemical and whole-organism assay approaches have recently gained prominence.
- Passive and active immunizations are emerging as key approaches to treating drug and substance abuse.
- Emerging areas of research include next generation technologies and target areas, such as three-dimensional models, automated animal-behavior monitoring, high-throughput discovery of synergistic drug-drug combinations, and increased appreciation of the role of epigenetics.

Table 1

Selected Neurotransmitter-Receptor HTS Assays.

Neurotransmitters	Receptors	HTS Assays	Reference
Acetylcholine	Muscarinic; Nicotinic	Phenotypic	[48], [49]
Norepinephrine	Adrenergic	Phenotypic	[40]
Dopamine	Dopamine; Neurotensin	Phenotypic	[40],[42,43]; [54]
Serotonin	Serotonin	Phenotypic and Target	[39,40]
Glutamate	NMDA; Metabotropic glutamate	Phenotypic	[51,52]
Opioids	delta, kappa, and mu	Phenotypic	[36,37]
Neuropeptides	y2; galanin; S; orexin; FosB	Phenotypic and Target	[62]; [64]; [67]; [69]; [77]

Table 2
Example Tool Compounds from Receptor cell-based HTS Assays

Name	Structure	Receptor	Plate Density	Reporter	References
ML326		Muscarinic acetylcholine	384	Ca ²⁺ (Fluo-4)	[129]
ML375		Muscarinic acetylcholine	384	Ca ²⁺ (Fluo-4)	[49]
ML381		Muscarinic acetylcholine (M5)	1,536	Ca ²⁺ (Fluo-4)	[48]
VU0463841		Metabotropic glutamate receptor subtype 5 (mGlu5)	384	Ca ²⁺ (Fluo-4)	[51]
ML138		Kappa Opioid Receptor	1,536	β-arrestin w/Luciferase	[130]
ML139		Kappa Opioid Receptor	1,536	β-arrestin w/Luciferase	[130]
ML140		Kappa Opioid Receptor	1,536	β-arrestin w/Luciferase	[130]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Name	Structure	Receptor	Plate Density	Reporter	References
ML190		Kappa Opioid Receptor	1,536	β-arrestin w/Luciferase	[130]
ML191		GPR55	384	GFP-labeled β-arrestin	[58]
ML192		GPR55	384	GFP-labeled β-arrestin	[58]
ML193		GPR55	384	GFP-labeled β-arrestin	[58]
ML321		Dopamine (D2)	1,536	Ca ²⁺ (Fluo-8)	[42]
ML297		GIRK Potassium Channel	384	Thallium Flux (Thallos-AM)	[57]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Name	Structure	Receptor	Plate Density	Reporter	References
ML154		Neuropeptide S	1,536	Ca ²⁺ (Fluo-4)	[67]
72		Orexin 1	96	calcium 5	[69]
32c		Sigma 1	96	[³ H] Radiolabeled	[131]
ML314		Neurotensin NTR1	1,536	GFP-labeled β-arrestin	[54]