Published in final edited form as:
Hum Genet. 2012 June ; 131(6): 941–957. doi:10.1007/s00439-011-1129-z.

Utility of genetically modified mice for understanding the neurobiology of substance use disorders

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Abstract

Advances in our ability to modify the mouse genome have enhanced our understanding of the genetic and neurobiological mechanisms contributing to addiction-related behaviors underlying substance use and abuse. These experimentally induced manipulations permit greater spatial and temporal specificity for modification of gene expression within specific cellular populations and during select developmental time periods. In this review, we consider the current mouse genetic model systems that have been employed to understand aspects of addiction and highlight significant conceptual advances achieved related to substance use and abuse. The mouse models reviewed herein include conventional knockout and knockin, conditional knockout, transgenic, inducible transgenic, mice suitable for optogenetic control of discrete neuronal populations, and phenotype-selected mice. By establishing a reciprocal investigatory relationship between genetic findings in humans and genomic manipulations in mice, a far better understanding of the discrete neuromechanisms underlying addiction can be achieved, which is likely to provide a strong foundation for developing and validating novel therapeutics for the treatment of substance abuse disorders.

Introduction

The profound negative impact that substance abuse exerts on the individual and society is staggering. While substance abuse disorders are generally considered preventable, research has repeatedly demonstrated that neurocircuits in the brain exhibit altered function in the addicted individual, potentially contributing to the maintenance of and/or relapse to the drug-taking habit (Azizian et al. 2009, 2010; Bolla et al. 2004; Ersche et al. 2005; London et al. 1990). Moreover, intrinsically altered function of brain systems may present the individual with an underlying predisposition to develop an addictive disorder when exposed to a drug of abuse. Advances in our understanding of the genetics of human drug addiction have been derived from genome wide association studies (GWAS), which have shown that

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various chromosomal regions and/or specific allelic genetic variations increase risk for dependence on tobacco (Berrettini et al. 2008; Schlaepfer et al. 2008; Sherva et al. 2008; Wang et al. 2009), cocaine (Dahl et al. 2005; Gelernter et al. 2005), opioids (Gelernter et al. 2006; Kreek et al. 2005), and alcohol (Batel et al. 2008; Sherva et al. 2010). However, data derived from GWAS analyses are correlative and may vary depending on populations examined (Blomqvist et al. 2000) or comorbid drug use within the sample population (Luo et al. 2005), potentially leading to conflicting and/or confounded results. Hence, it is important that genetic association analyses be accompanied by in-depth laboratory studies that can provide more direct support for the involvement of candidate genes identified in GWAS for vulnerability to develop substance use and abuse. In this context, genetically modified mice are useful, as genes of interest can be directly manipulated and their contribution to drug consumption or drug seeking behaviors directly assessed. Genetically modified mice are also beneficial to discern discrete neurobiological mechanisms—neurocircuitry, neurotransmitters, receptors, signaling pathways, modulatory substrates—contributing to drug use and abuse, insights that are far more difficult to achieve in human laboratory experiments. When data derived from animal models support the involvement of candidate genes identified in human genetic association analyses, this convergent evidence can substantially enhance our understanding of the genetics of addiction and validate novel targets for therapeutic development (Brody et al. 2006a, b; Wong et al. 2006).

In addition to supporting human GWAS studies, genetically modified mice can provide considerable insights into the basic neurobiological mechanisms of addiction. Given recent technical advances in our ability to manipulate the mouse genome, mice have increasingly become a more versatile model system with considerable spatial and temporal resolution in terms of controlling gene expression. Importantly, the human and mouse genomes exhibit a high degree of homology, thus providing a strong basis for inference of gene function across these species. In this regard, mutant mice have been used to investigate the role of genes in drug-associated behaviors, such as drug reinforcement, reward, withdrawal and relapse-like behavior. If the mouse exhibits an altered addiction-related phenotype, the importance of specific candidate genes, and the molecular mechanisms through which they may act, can be directly examined. Mutant mice can also be employed to identify novel cell-type specific biomarkers that could provide benefits for future drug targets. Given the international efforts to create genetically engineered mice for the majority of the protein-coding genes (International Mouse Knockout Consortium 2007; Skarnes et al. 2011; Tate and Skarnes 2011) and the increasing numbers of mouse lines becoming available (GENSAT 2011; Gong et al. 2007), the genetically engineered mice provide a unique opportunity for researchers to delineate the genetics underlying multifaceted traits (Aylor et al. 2011) and as such, hold great promise for future investigations into the mechanisms underlying drug addiction.

In this article, we provide an overview of available strategies to manipulate the mouse genome, consider recent data highlighting how manipulation of the mouse genome has provided new insights into the genetics of substance abuse disorders, and discuss how genetically modified mice can support human GWAS studies. In general, the individual research hypothesis and question largely dictate the model that is most useful for a given experiment. For example, an individual may be interested in examining gene function in the absence of the gene (e.g., knockout model) or identifying protein expression in the brain (e.g., reporter mice). Further considerations may also incorporate the importance of genetic manipulation given the (1) developmental stage, (2) timing with regards to experimental design, (3) cell-type specificity, (4) brain area specificity, (5) level of gene expression desired (e.g., elimination vs. reduction), and (6) limitations of a specific model (e.g., if examining reproductive function, administering tamoxifen in an inducible model may introduce off-target effects). Thus, the benefits and inherent limitations of each model will

also be reviewed. Further, examples of convergence between human GWAS studies and mouse model systems are provided that highlight the potential of genetically modified mice to identify novel targets for therapeutic development.

Conventional knockout mice

Gene trapping and gene targeting are the most commonly utilized methods to introduce site-specific genetic mutations into the genome of mouse embryonic stem cells [e.g., see reviews (Guan et al. 2010; Koentgen et al. 2010)]. The conventional knockout mice constitutively lack expression of the targeted gene throughout their entire lifespan (Fig. 1A). Our laboratory has recently utilized conventional knockout mice to define the role of the α5 nicotinic acetylcholine receptor (nAChR) subunit in nicotine reinforcement (Fowler and Kenny 2011; Fowler et al. 2011). We found that mice with null mutation in the α5 nAChR subunit gene (α5 knockout) intravenously self-administer dramatically greater quantities of nicotine, and demonstrate increased motivation to seek and obtain the drug, compared with their wildtype littermates. This addiction-like phenotype in the α5 knockout mice is most apparent when higher unit doses of the drug are available for consumption (Fowler et al. 2011). Importantly, recent findings in humans have shown that allelic variation in the *CHRNA3-CHRNA5-CHRNB4* gene cluster, which encodes the α3, α5 and β4 nAChR subunits respectively, increases vulnerability to tobacco dependence and smoking-associated diseases (Bierut et al. 2008; Thorgeirsson et al. 2008). In particular, smoking-associated risk alleles of the *CHRNA5* gene give rise to mature α5 subunits that result in sub-functional nAChRs compared with wildtype α5-containing nAChRs (Bierut et al. 2008). Hence, the addiction-like phenotype in α5 knockout mice is very similar to the increased addiction vulnerability detected in individuals that carry *CHRNA5* risk alleles. Since the α5 nAChR subunits exhibit a restricted expression profile in the brain, with particularly high density in the medial habenula (MHb) and its major site of projection, the interpeduncular nucleus (IPN) (Marks et al. 1992), the role of this pathway in the enhanced nicotine consumption in the knockout mice was examined. We found that the α5 knockout mice exhibited a significant reduction in nicotine-induced activation of the IPN, as measured by Fos immunoreactivity, compared with wild-type mice (Fowler et al. 2011). Next, we employed a virus-based approach to "rescue" α5 nAChR subunit gene expression in the MHb-IPN pathway in α5 knockout mice and assessed the effects of such site-specific re-expression on nicotine intake in the knockout mice. We found that lentivirus-mediated delivery of the α5 nAChR subunit gene into the MHb-IPN pathway, which permitted discrete site-specific investigation of gene function in a spatially and temporally controlled manner, reversed the enhanced nicotine consumption typically found in the α5 knockout mice (Fowler et al. 2011). These data show that knockout mice can demonstrate a phenotype similar to that detected in humans with genetic variation in the same gene. These findings also highlight the utility of knockout mice to investigate the functional relevance of brain pathways underlying addiction-related behaviors with experimental manipulations not permissible in human subjects.

While knockout mice are a useful platform for investigating the contribution of discrete genes to addiction-like behaviors, limitations of this approach must be taken into consideration. First, the knockout mice have constitutively lacked gene expression throughout development, potentially leading to compensation via alteration in the expression of other genes. Currently, it is unknown whether specific mutations are particularly prone to compensation, in part because an exhaustive investigation into all of the possible compensatory mechanisms is not routinely undertaken for each genetically engineered mouse generated. However, an increasing array of tools has begun to permit this issue to be experimentally addressed in a more time-and cost-effective manner. Strategies in which the genetic manipulation may be rescued (e.g., lentiviral or adenoviral insertion of the gene in a

knockout model) strengthen the conclusion that a specific gene is necessary for an observed phenotype, as demonstrated above with the α5 nAChR subunit lentiviral 'rescue' that normalized nicotine intake in the α5 knockout mice (Fowler et al. 2011). Second, gene expression is eliminated throughout the entire organism and not just in brain circuitries or neuronal populations that may be of particular interest. Hence, opposing actions of the gene in different brain sites could potentially result in a misleadingly low (or even undetectable) contribution of the gene to the behavior of interest, or result in alterations in other biological processes that indirectly influences the expression of addiction-like behaviors in a non-specific manner. Third, species differences in the background genetic profile may lead to a strain-specific phenotype. To address these potential confounding factors, we sought to manipulate α5 nAChR subunit expression specifically in the MHb-IPN pathway during adulthood in a different species in which the α5 subunit gene was not constitutively deleted throughout development. Specifically, we used a lentiviral-mediated gene transfer approach to knockdown α5 nAChR subunit expression discretely in the MHb of adult rats (Fowler et al. 2011). Using this approach, we recapitulated the addiction-like phenotype in adult knockdown rats similar to that observed in the α5 knockout mice, reflected in increased nicotine self-administration attributable to reduced expression of α5 nAChR subunits in the MHb-IPN pathway (Fowler et al. 2011). These findings were then extended to demonstrate that in rats, virus-mediated knockdown of α5 nAChR subunits in the MHb-IPN tract did not alter the reward-enhancing properties of lower nicotine doses, but greatly attenuated the reward-inhibiting (i.e., aversive) effects of higher nicotine doses, and lidocaine-induced inactivation of the MHb or IPN greatly increased nicotine self-administration, particularly at high unit doses of the drug (Fowler et al. 2011). Together, these data reveal that the behavioral phenotype found in the α5 knockout mice was not due to developmental compensatory changes or a species-specific phenotype, but rather the enhanced nicotine consumption could be directly attributable to decreased expression of α5 nAChRs in the MHb-IPN tract. Moreover, using knockout mice combined with virus-mediated gene transfer technology, it is possible to identify and validate neuroanatomical substrates within which genes of interest may act to influence addiction-related behavior.

With their increasing availability (International Mouse Knockout Consortium 2007; Skarnes et al. 2011; Tate and Skarnes 2011), knockout mouse lines are being increasingly utilized to examine a variety of biological mechanisms that may be impacted by drugs of abuse and contribute to addiction. Further, some groups have bred mice that exhibit more than one gene knockout to further define the concerted effects of their simultaneous deletion, or to investigate the potential opposing actions of different genes, on addiction-related behaviors (Exley et al. 2011; Sora et al. 2001). Since the knockout model has been the most commonly employed mutant model thus far, we refer the reader to recent, extensive reviews. These papers detail the progress utilizing knockout mice to determine the neurobiological mechanisms involved with nicotine (De Biasi and Dani 2011; Tuesta et al. 2011), psychostimulants (Sora et al. 2010), opioids (Kreek et al. 2005; Trigo et al. 2010), and alcohol (Crabbe et al. 2006).

Conventional knockin mice

As opposed to disrupting gene function, an alternate gene targeting approach can be applied in which a gene is altered with the goal of (1) expressing the protein product with altered function than the wildtype product, (2) over-expression of a gene product, or (3) expression in cells that do not normally express the gene of interest. For example, as an alternative to the dopamine transporter (DAT) knockout mice, transgenic mice were created to alter the amino acid residues in the DAT gene that are important for cocaine binding (Chen et al. 2006). With this modification, the transporter becomes relatively insensitive to cocaine inhibition but can still function to maintain a normal level of dopamine reuptake. Unlike

wildtype mice, the DAT cocaine insensitive mice were unable to establish cocaine-induced conditioned place preference (CPP) or cocaine self-administration, and also lacked some cocaine-induced behaviors (Chen et al. 2006; Thomsen et al. 2009; Tilley and Gu 2008; Tilley et al. 2009). A benefit of the knockin strategy is that the allele tends to exert a dominant phenotype, as opposed to the more recessive phenotype evidenced with a null mutation allele (Koentgen et al. 2010). The gene 'knockin' may be achieved by inserting the gene into the coding sequence of the endogenous site via mini cDNA or inversion methods with the Cre/loxP system in embryonic stem cells (Koentgen et al. 2010) (Fig. 1B). Further, by selectively inserting the gene at the *ROSA26* locus, ubiquitous overexpression of the gene may be achieved (Heyer et al. 2011). Alternatively, viral vectors may insert the transgene into endogenous gene locus at the initiation codon or the 3′ non-translated region in embryonic stem cells, and this requires that the transgene contains an open reading frame preceded by an internal ribosome entry site (IRES) (Koentgen et al. 2010). The knockin method can also be used to generate a hypomorphic mouse with altered or reduced expression of a gene. These mutations may be beneficial when complete knockout of a gene product induces prenatal lethality or a severe behavioral phenotype. In the conditional dopamine-deficient (DD) mouse, a floxed Neo gene is inserted into the first intron of the tyrosine hydroxylase (TH) gene, thereby inhibiting its functional expression and resulting in dramatically reduced levels of dopamine in the brain (Hnasko et al. 2006). These mice exhibit hypophagia, bradykinesia, altered reward-directed behaviors, and disrupted active avoidance learning (Darvas et al. 2011; Hnasko et al. 2006; Robinson et al. 2007). Injection of a canine adenovirus to express Cre recombinase permitted restoration of normal TH gene expression and reversed the behavioral phenotype (Darvas et al. 2011; Hnasko et al. 2006; Robinson et al. 2007). Interestingly, the DD mice form a cocaine-induced CPP which is not altered with administration of a dopamine antagonist but rather with an SERT inhibitor (Hnasko et al. 2007), thus implicating serotonin in cocaine reward under conditions of reduced dopamine transmission.

The nAChRs regulate the actions of nicotine in the brain, with the $\alpha4\beta2$-containing nAChRs being among the most abundantly expressed. As such, the $\alpha4\beta2$-containing nAChRs have been the most intensively studied for their involvement in the rewarding and reinforcing effects of nicotine, particularly because of their dense expression within the mesocorticolimbic dopamine system. To investigate the involvement of the $\alpha4$ nAChR subunit, Tapper et al. (2004) engineered knockin mice with a single point mutation (Leu9′Ala) in the putative pore-forming domain. In vitro, the mutant $\alpha4$-containing receptors demonstrate increased agonist binding, nicotine-induced neuronal responses, and functional up-regulation. Behaviorally, the mutant mice developed a CPP for low doses of nicotine that was without effect in their wildtype counterparts (Tapper et al. 2004). Furthermore, the $\alpha4$ hypersensitive knockin mice have been utilized to demonstrate a role for $\alpha4$-containing nAChRs in the effects of varenicline (Chantix) on alcohol consumption (Hendrickson et al. 2010), dopamine D2 receptor modulation of motor behavior (Zhao-Shea et al. 2010), and the actions of nicotine on dopaminergic neurons in the posterior ventral tegmental area (VTA) (Zhao-Shea et al. 2011). A second line of $\alpha4$ hypersensitive knockin mice has also been created to contain a different point mutation (Ser248′Phe), and these mice demonstrate increased self-administration at a lower dose of nicotine (Cahir et al. 2011).

Given the identification of human allelic variation that may increase vulnerability to substance abuse disorders, modification of a mouse gene to reflect a specific human SNP or insertion of a humanized gene into a mouse model can provide insight into in vivo allelic receptor function in a more subtle manner than entire gene ablation or insertion. A single nucleotide polymorphism in the μ-opioid receptor gene, A18G, has been extensively implicated in substance use and dependence [for review, see (Mague and Blendy 2010)].

Mague et al. (2009) developed a transgenic mouse with an equivalent point mutation in the mouse gene, A112G. The knockin mice exhibited an attenuation of morphine-induced hyperlocomotion, antinociceptive responses, and did not develop sensitization. Interestingly, development of a place preference for morphine and place aversion for morphine withdrawal could be established in male, but not female, knockin mice (Mague et al. 2009), suggesting that alternate genes and/or gonadal steroid hormones may interact with A112G allele to regulate morphine reward. Finally, a humanized cytochrome P4502E1 (CYP2E1) gene was knocked into mice lacking the mouse P4502E1 gene (CYP2E1 KO mice). Whereas the CYP2E1 KO mice demonstrated an attenuated response to chronic ethanol-induced liver injury, the humanized CYP2E1 knockin mice developed fatty livers and expressed markers of oxidant stress similar to wildtype mice (Lu et al. 2010), demonstrating the importance of the human gene in the effects of ethanol in the liver. These studies provide additional validation of the similarity of mouse and human gene function and highlight the relevance of data obtained from mouse models to the human condition.

Conditional knockout mice

The ability to conditionally modify gene expression in a spatially and temporally controlled manner presents a distinct experimental advantage over constitutive knockout or knockin technologies, as this permits controlled manipulation of cellular function within the brain. As such, the Cre-loxP system affords the ability to specifically modify the genome for a variety of organisms (Meijer et al. 1998; Odell et al. 1990; Sauer and Henderson 1989; Wu et al. 2011). Cre recombinase was initially discovered in P1 bacteriophage as a mechanism to facilitate replication of genomic DNA during reproduction (Sauer and Henderson 1988; Sternberg et al. 1981). Cre catalyzes recombination between two loxP sites flanking a genomic sequence of interest, resulting in removal, inversion or chromosomal translocation of the DNA segment (Nagy 2000). The loxP site consists of a core sequence and two flanking inverted repeats (Nagy 2000). To modify a gene of interest, two different transgenic strains need to first be created: (1) mice with two loxP sites flanking a DNA segment of interest (Flox mice), and (2) mice with Cre recombinase under the control of a selected promoter (Cre mice) (Nagy 2000). When these mice are bred together, conditional knockout is achieved as the Cre enzyme recombines the floxed gene, thus resulting in altered gene expression in a cell-specific manner (Fig. 1C). Similar to the Cre/loxP system is the Flp/FRT method in which Flp recombinase derived from yeast binds to FRT sites flanking the gene of interest (Koentgen et al. 2010). The promoter for the transgene expressing Cre (or Flp) determines whether the expression will be generalized or cell-type specific. Furthermore, by employing RNA interference in conjunction with Cre/loxP, a vector may be created to permit independent modulation of two different genes (Steuber-Buchberger et al. 2008). In addition to providing cell- or tissue-specific expression, another advantage of selecting specific promoters is that they may permit altered expression during a given developmental stage in accordance with the gene's endogenous expression pattern. For example, mice with Cre under a CaMKIIα promoter were found to exhibit a restricted expression profile in the forebrain that only becomes evident postnatally (Akbarian et al. 2002; Minichiello et al. 1999). Thus, conventional gene knockouts resulting in prenatal lethality can be more appropriately investigated by permitting gene modification at a later developmental stage; this approach has proven successful for investigations of brain-derived neurotrophic factor (BDNF) (Akbarian et al. 2002) and cAMP response element-binding protein (CREB) (Rudolph et al. 1998). An alternate method is to inject a virus expressing Cre into a specific brain region in the postnatal or adult animal (Graham et al. 2009; Parker et al. 2011). Instead of the global knockout of all cells containing the gene throughout the brain, this method allows for selective cell-type and brain region specific knockdown.

Several limitations should be noted with conditional modification of gene expression. First, transgene integration may result in varied levels and/or mosaic pattern of gene expression, or insertional mutagenesis if integrated into an endogenous gene (Nagy 2000; Sacca et al. 2010). Next, transgene expression may vary or become undetectable across generations (Sacca et al. 2010; Sauer 1998; Sutherland et al. 2000), and excessive loxP integration sites can result in chromosome loss (Lewandoski and Martin 1997). Further, the targeted genes are likely not completely eliminated from expression (Lambert 2009), which may result in a low residual level of endogenous gene expression. Depending on the size of the gene, the loxP sites also may not be able to flank the entire region, which could result in the production of an abnormal gene product with altered function (Morice et al. 2004). More advanced methods have been developed in an attempt to eliminate or reduce these problems, including bacTRAP (Doyle et al. 2008; Heiman et al. 2008), variations of Flp recombinase (Kranz et al. 2010; Wu et al. 2009), and short hairpin RNA (shRNA) transgenic mice (Hitz et al. 2009). Furthermore, by combining multiple genetic modification techniques, such as Cre/loxP and Flp/FRT, an intersectional strategy can be achieved to permit conditional regulation of gene expression and function (Dymecki et al. 2010). This ‘conditional intersectional genetics’ method has been recently utilized to demonstrate the importance of serotonergic neurons in homeostatic function (Ray et al. 2011). In this study, the mouse model was generated utilizing Cre and Flp transgenics to conditionally induce hyperpolarization of serotonergic neurons following administration of the synthetic ligand clozapine-*N*-oxide (CNO) (Ray et al. 2011). This mouse line was created by breeding mice expressing: (1) a $G_{i/o}$ protein-coupled receptor (DREADD, hM_4D) engineered to bind CNO and (2) Cre under the *Slc6a4* (serotonin transporter) gene.

In recent years, conditional knockouts have been successfully employed to investigate the mechanisms of action of various drugs of abuse. McGranahan et al. (2011) demonstrated that $\alpha 4$-containing nAChRs on dopaminergic neurons are involved in nicotine reward and anxiety. By crossing mice with loxP sites surrounding the $\alpha 4$ nAChR subunit gene and DAT-Cre mice ($\alpha 4^{DATCre}$), selective deletion of $\alpha 4$ nAChR subunits in dopaminergic neurons was achieved. These mice demonstrated an impaired CPP for nicotine, but not cocaine, as well as enhanced nicotine-induced hypolocomotion and attenuation of nicotine-induced anxiolytic effects (McGranahan et al. 2011). Interestingly, nicotine-induced CPP was also disrupted in mice with a conditional knockout of the NMDA receptor NR1 subunit gene, *Grin1*, in dopaminergic neurons ($NR1^{DATCre}$) (Wang et al. 2010). Together these data suggest that $\alpha 4$* nAChRs and NMDA receptors may interact in dopaminergic neurons to regulate nicotine reward.

Cocaine reward and sensitization have also been assessed with conditional knockout mice to selectively eliminate receptor and signaling proteins. BDNF and its receptor, TrkB, were manipulated in mice, in which with each gene was “floxed” (Graham et al. 2009). Site-specific administration of a Cre adenovirus permitted knockdown during adulthood since embryonic knockout results in prenatal lethality. Decreased expression of BDNF in the nucleus accumbens (NAc) or VTA inhibited the formation of a cocaine-induced CPP (Graham et al. 2009). Similarly, TrkB knockdown prevented a CPP for cocaine and also reduced reinforcement, as evidenced by a downward shift in the dose–response curve for intravenously self-administered cocaine infusions (Graham et al. 2009). Conversely, selective deletion of dopamine D2 autoreceptors in $DrD2^{Dat/IRES\text{-}Cre}$ mice resulted in enhanced cocaine-induced locomotion, CPP and motivation to obtain food reward (Bello et al. 2011). Enhanced cocaine-induced locomotion has also been found when dopaminergic neurons lack muscarinic M_4 receptors in $M_4^{Drd1a-Cre}$ mice (Jeon et al. 2010). Knockdown of the glucocorticoid receptor (GR) in neurons altered cocaine-induced gene expression, suppressed locomotor sensitization and decreased cocaine self-administration as

demonstrated in $GR^{nestin\text{-}Cre}$ mice (Deroche-Gamonet et al. 2003; Izawa et al. 2006). Acquisition and motivation to self-administer cocaine were attenuated in mice lacking CREB in the cerebral cortex of $Creb1^{Emx\text{-}1\text{-}Cre}$ mice (McPherson et al. 2010), although differences were not found for cocaine-induced CPP in mice lacking CREB generally throughout the brain ($Creb1^{Nestin\text{-}Cre}$ mice) (Valverde et al. 2004). Finally, the lack of Ca2+/calmodulin-dependent protein kinase IV (CAMKIV) in dopaminergic neurons enhanced the expression of a cocaine-induced CPP and reinstatement of CPP after extinction in $CamkIV^{Drd1a\text{-}Cre}$ mice (Bilbao et al. 2008). Based on these findings, the authors were then able to establish an association between a single nucleotide polymorphism (SNP) in the *CamkIV* gene and cocaine addiction in humans (Bilbao et al. 2008), highlighting the translational possibilities of data derived from the conditional knockout mice.

Glutamatergic signaling has also been investigated in conditional knockout mice in response to cocaine. Mice with disrupted expression of the vesicular glutamate transporter 2 in dopaminergic neurons ($VGLUT2^{DAT\text{-}Cre}$) exhibit decreased glutamate release from dopaminergic terminals in the NAc (Hnasko et al. 2010; Stuber et al. 2010) and dopamine storage and release deficits in the ventral striatum (Hnasko et al. 2010). Behaviorally, $VGLUT2^{DAT\text{-}Cre}$ mice show reduced cocaine-induced hyperlocomotion (Hnasko et al. 2010). Next, by comparing mice lacking cannabinoid type 1 (CB1) receptors in principal forebrain neurons ($CB1^{CaMKII\alpha\text{-}Cre}$) or in GAB-Aergic neurons ($CB1^{Dlx5/6\text{-}Cre}$), presynaptic CB1 receptors were found to mediate glutamatergic synaptic transmission in forebrain principal neurons (Domenici et al. 2006) and cocaine-induced phosphorylation of extracellular signal-regulated kinase (ERK) in the dorsal striatum and NAc (Corbille et al. 2007). Glutamate receptor subtypes such as metabotropic glutamate 5 receptors (mGluR5) and *N*-methyl-D-aspartate (NMDA) receptors have also been investigated. Transgenic mice were developed utilizing short hairpin interfering RNA (shRNA) to knockdown mGluR5 in cells expressing the dopamine D1 receptor (Novak et al. 2010). A construct to express microRNAs targeting mGluR5 mRNA was inserted after the translational start of the D1R gene in BAC to produce the $mGluR5^{KD\text{-}D1}$ mice. Their data suggest that while the mGluR5 signaling in D1R expressing neurons is not important for cocaine self-administration, they do appear to play a role in cue-induced reinstatement (Novak et al. 2010). Next, floxed NR1 mice were crossed with RGS9-Cre mice to permit NMDA knockdown specifically in striatal cholinergic interneurons, and the $NR1^{RGS9\text{-}Cre}$ mice exhibited deficits in the formation of a CPP for cocaine (Agatsuma et al. 2010). However, conflicting reports have emerged for the role of NMDA receptors based on different $NR1^{DATCre}$ mouse lines. In two reports, $NR1^{DAT\text{-}Cre}$ mice exhibited a normal CPP to cocaine (Engblom et al. 2008; Luo et al. 2010), whereas another study was unable to demonstrate a cocaine-induced CPP in $NR1^{DAT\text{-}Cre}$ mice (Zweifel et al. 2008). In addition, one study found no deficits in cocaine sensitivity following sensitization and reinstatement of CPP after extinction in $NR1^{DAT\text{-}Cre}$ mice (Luo et al. 2010), whereas the other two studies established differences in $NR1^{DAT\text{-}Cre}$ mice compared to controls (Engblom et al. 2008; Zweifel et al. 2008). The reasons for such discrepancies among mouse lines are unclear, but may be due to the different approaches utilized to create each mouse line. For example, the line generated by Zweifel et al. (2008) was heterozygous for the genes for DAT and NR1, except in the dopaminergic cells lacking both *Grin1* alleles for NR1. Engblom et al. (2008) produced mice with BAC vector and wildtype controls lacked the BAC transgene, so the integration site or expression pattern may have introduced potential problems (see discussion of BAC methodology below). In contrast, Luo et al. (2010) created their BAC transgenic mice using an IRES sequence to target the stop codon at the DAT gene locus to allow for transgenic gene expression through both alleles. The IRES is also thought to reduce integration site and expression pattern problems associated with the more traditional BAC methodology (Luo et al. 2010). A gene re-expression approach may be warranted with the $NR1^{DAT\text{-}Cre}$ mice to determine if the

behavior can be 'rescued' with reinstated site-specific gene expression and resolve the contradictory findings described above [e.g., see (Parker et al. 2011)].

In addition to nicotine and cocaine, conditional knockouts are beginning to be employed for other drugs of abuse. Amphetamine sensitization was impaired with conditional knockdown of NMDA receptors in dopaminergic D1 receptor neurons in $NR1^{Drda1\text{-}Cre}$ mice (Beutler et al. 2011) and of CB1 receptors in forebrain neurons in $CB1^{CamKII\alpha\text{-}Cre}$ mice (Corbille et al. 2007), but not with knockdown in both D1 and D2 receptor neurons in $NR1^{DAT\text{-}Cre}$ mice (Beutler et al. 2011). In contrast, enhanced amphetamine sensitization was found with elimination of adenosine A_{2A} receptors in the forebrain in $A_{2A}^{CaMKII\alpha-Cre}$ mice (Bastia et al. 2005) and muscarinic M_4 receptors in dopaminergic neurons in $M_4^{Drd1a-Cre}$ mice (Jeon et al. 2010). By comparing multiple conditional mouse lines, decreased behavioral and autonomic responses for Δ^9-tetrahydrocannabinol (THC) were found in mice lacking the CB1 receptor in forebrain neurons ($CB1^{CamKII\alpha\text{-}Cre}$ mice), cortical glutamatergic neurons ($CB1^{NEX\text{-}Cre}$ mice), and dopaminergic neurons ($CB1^{Drd1a\text{-}Cre}$ mice), but not in GABAergic neurons ($CB1^{Dlx5/6\text{-}Cre}$ mice) (Monory et al. 2007). Finally, conditional knockouts have been used to investigate the addiction-related effects of opiates. Attenuation of the somatic signs of morphine withdrawal was found in mice lacking expression of neurotrophin 3 or CREB in general neurons ($NT\text{-}3^{Nestin\text{-}Cre}$ mice, $Creb1^{Nestin\text{-}Cre}$ mice, respectively) (Akbarian et al. 2001; Valverde et al. 2004), or BDNF specifically in the forebrain ($BDNF^{CamKII\alpha\text{-}Cre}$ mice) (Akbarian et al. 2002). Interestingly, opiate-mediated expression of cAMP and tyrosine hydroxylase was altered in the locus coeruleus in $BDNF^{CamKII\alpha\text{-}Cre}$ mice (Akbarian et al. 2002), demonstrating the importance of projection sites for BDNF-mediated drug-induced plasticity. Although serotonergic neurons may be affected by opiates in the locus coeruleus, these neurons do not appear to be essential for morphine tolerance and reward. Removal of the transcription factor Lmx1b gene results in selective death of serotonergic neurons in the hindbrain due to their expression of Pet1 (Zhao et al. 2006, 2007a). Thus, $Lmx1b^{Pet1\text{-}Cre}$ mice exhibit a conditional elimination of serotonergic neurons during development; however, these conditional knockout mice did not display deficits in morphine-induced CPP or tolerance, whereas analgesia induced by a κ-opioid receptor agonist and antidepressant drugs was attenuated (Zhao et al. 2007a, b).

Transgenic mice

As opposed to selective site-directed gene targeting, transgenic mice can be created by injecting exogenous DNA, either as DNA plasmid or a bacterial artificial chromosome (BAC) vector, into a fertilized egg (Collins et al. 2007). BACs are circular plasmid DNA molecules derived from *E. coli* (Shizuya et al. 1992). Large DNA inserts may be incorporated into the BAC vector to produce optimal levels of the transgene through autonomous replication (Shizuya et al. 1992). Since all regulatory sequences for the expression domain are localized within the BAC, this method is considered superior to standard constructs and should result in expression patterns similar to the endogenous gene (Giraldo and Montoliu 2001; Heintz 2001). However, consideration must be taken for the limitations of the method. As opposed to the specific mutation obtained with the conventional knockin method, this transgenic approach produces random insertion into the genome and can often result in multiple copies introduced at more than one site (Fig. 1D), thus necessitating the need to screen and characterize subsequent generations of mice. Furthermore, while the endogenous gene remains unaltered with exogenous gene insertion, regulatory mechanisms within the cell may alter endogenous gene expression, unless the insertion is created in a knockout mouse for the gene of interest. Next, expression of the introduced gene may be altered throughout development or manipulated during a certain time period dependent on the gene expression profile of the promoter (Rios et al. 2006).

Thus, resulting behavioral phenotype(s) may reflect altered gene expression that was induced at a prior developmental stage, rather than during the period examined during the experiment. Similar to the conventional knockout and knockin methods, alternate neural mechanisms could also exhibit a compensatory response (Rios et al. 2006).

This approach has been recently utilized to genetically engineer mice to overexpress a protein of interest for investigations into drug-related mechanisms. For example, to investigate the role of the DAT in cocaine reward, Donovan et al. (1999) created a transgenic mouse to overexpress DAT under the tyrosine hydroxylase promoter (ThDAT mice). ThDAT mice demonstrated enhanced cocaine reward, as assessed by CPP, but did not exhibit differences in cocaine-induced hyperlocomotion (Donovan et al. 1999). Another group generated mice with a hypersensitive α6 nAChR subunit containing a single point mutation in the putative pore-forming domain (Leu9′Ser) (Drenan et al. 2008). The α6 hypersensitive mice exhibited enhanced nicotine-induced hyperlocomotion compared with wildtype mice, and when they were subsequently crossed with α4 knockout mice, the α6 hypersensitive/α4 knockout mice demonstrated greatly diminished responses to the stimulant effects of nicotine (Drenan et al. 2010). Thus, by combining both the conventional knockout and transgenic approaches, the authors were able to demonstrate that co-expression of both the α4 and α6 nAChR subunits is important for the stimulant effects of nicotine. Frahm et al. (2011) utilized a transgenic mouse with targeted overexpression of the β4 nAChR subunit (termed Tabac mice). The Tabac mice drank less water containing nicotine and exhibited a nicotine-induced place aversion at nicotine concentrations that did not trigger aversion in wildtype mice (Frahm et al. 2011). In this same study, the investigators used a lentivirus vector to express a risk allele of the α5 subunit gene associated with increased tobacco use in humans (D398N) in the Tabac mice. This manipulation, in essence “humanizing” the mice, increased the resistance of the Tabac mice to the aversive properties of nicotine, suggesting that overexpression of the β4 subunit along with expression of the humanized D398N variant of the α5 subunit in the habenula are sufficient to restore aversive nicotinic conditioning. It will be interesting in further studies to independently examine the function of the β4, as well as the α3, nAChR subunits in genetically engineered mice to determine their specific contributions to regulating nicotine reinforcement.

In addition to modifying gene expression, numerous transgenic mice are being generated with the BAC method to examine the expression profile of a gene of interest. In these reporter mice (Fig. 1D), the coding sequence for the target gene is replaced with a LacZ or fluorescent reporter and promoter-driven selection cassette (Collins et al. 2007). Identification of cell-specific gene expression within the tissue can be examined through visualization of β-galactosidase (with LacZ) or fluorescence-specific laser excitation. This method is particularly attractive for proteins not effectively identified with the currently available antibodies for immunocytochemistry. Another beneficial use of the Cre/loxP method is to verify site-specific gene expression for a Cre transgenic strain. In this circumstance, reporter mice containing both a floxed gene with a stop codon and a fluorescent, lacZ or luciferase reporter are employed (Boer et al. 2010; Lambert 2009). In particular, mice with the loxP sites surrounding the *ROSA26* gene are good candidates for this type of validation based on the generalized expression pattern of the gene and conserved genomic structure across species (Gong et al. 2007; Irion et al. 2007). As such, recombinase activity can be verified histologically with *ROSA26*-lacZ mice that have been (1) injected with a Cre adenovirus (Berton et al. 2006), or (2) crossed with a Cre mouse line (Wang et al. 2010; Zweifel et al. 2008). By utilizing the BAC method to insert Cre with a cell-type specific promoter (Gong et al. 2007), increasing numbers of BAC-Cre recombinase driver mouse lines are being generated. To date, the GENSAT (Gene Expression Nervous System

Atlas) project provides information for 161 Cre lines and lists numerous others currently in production (GENSAT 2011).

Based on the importance of the dopaminergic mesolimbic pathway in the actions of many drugs of abuse, it is not surprising that dopamine D1 (*Drd1*) and D2 (*Drd2*) receptor reporter lines have been most extensively studied thus far (Gertler et al. 2008; Kreitzer and Malenka 2007; Shen et al. 2008). Further, since striatal medium spiny neurons express either D1 or D2 receptors, these mice have permitted dissociation between these two cellular populations. For example, increased spine density following chronic cocaine treatment was found in neurons expressing both the D1 and D2 receptors, but only the D1-containing neurons maintained a stable level of density during withdrawal (Lee et al. 2006). Interestingly, the increased spine density was associated with elevated ΔFosB expression for both the *Drd1*-EGFP and *Drd2*-EGFP neurons (Lee et al. 2006). Chronic cocaine also induced an increase in NFκB-dependent transcription, as demonstrated with NFκB-lacZ mice (Russo et al. 2009). Further, cocaine withdrawal has been shown to increase the number of vasopressin (AVP) neurons in the paraventricular nucleus in AVP-EGFP mice (Zhou et al. 2011). Finally, pro-opiomelanocortin (POMC)-GFP mice have been utilized to identify neurons involved in nicotine-mediated feeding effects. GFP-labeled POMC neurons were identified in the arcuate nucleus of the hypothalamus for electrophysiological recording, and the authors eloquently demonstrated dose-dependent increases in spontaneous firing of these neurons with nicotine administration (Mineur et al. 2011).

Although these mice hold great promise for identification and manipulation of specific cell types within the brain, some transgenic lines also show ectopic or partial expression likely resulting from the transgene insertion site or transient expression of the gene during development (Gong et al. 2007). While verification of transgene expression can be performed, it requires prior knowledge of the endogenous pattern of protein expression, which may be unknown for some genes. Further, a recent report has demonstrated D2 receptor overexpression and altered electrophysiological and behavioral responses in *Drd2*-EGFP mice, while the *Drd1*-EGFP mice were more similar to the wildtype controls in baseline receptor gene expression (Kramer et al. 2011). The authors suggest receptor overexpression may have been due to the BAC insertion site, multiple copies of the transgene, or the BAC clone Ttc12 gene product (Kramer et al. 2011). Thus, investigations with BAC reporter mice should first verify receptor expression relative to wildtype levels prior to further experimental manipulation and/or inference of experimental findings. It may also be beneficial to use mice with only one copy of the transgene, or to create multiple founder lines and utilize the line that best represents the wildtype expression level and pattern (Kramer et al. 2011). Alternatively, the conventional knockin approach may be utilized to insert the fluorescent gene (e.g., EGFP) into the genome with greater site specificity.

Inducible transgenic mice

Several methods have been employed to produce an inducible loss or gain of function for a gene of interest. In one such approach, bitransgenic mice are created from two founder lines that (1) express the gene of interest under the control of a tetracycline-regulated promoter (TetOp) and (2) carry a transgene encoding a specific promoter (e.g., neuron-specific enolase and NSE) driving expression of the tetracycline transactivator (tTA) (Fig. 1E). Gene expression is then manipulated at a certain point of time upon administration of the inducing agent doxycycline which can be provided in the drinking water. Further, removal of doxycycline can subsequently reverse the gene silencing to reinstate gene expression. In a complementary method, the TetOp promoter may be used to drive Cre expression in a foxed mouse model; when doxycycline is administered under these conditions, Cre mediates

recombination of the floxed gene, leading to a gene knockout condition. The TetOp-tTA approach has been used to demonstrate that overexpression of ΔFosB increases cocaine-induced locomotion and CPP (Kelz et al. 1999), enhances acquisition and motivation to self-administer cocaine (Colby et al. 2003), and represses the histone methyltransferase G9a similar to that found with repeated cocaine administration (Maze et al. 2010). CREB overexpression was also shown to attenuate cocaine-induced locomotion (Sakai et al. 2002). In addition to inserting a gene similar to the endogenous allele, a mutant gene may be created to produce an altered gene product or a dominant-negative protein that acts as an antagonist to the endogenous protein (Dinieri et al. 2009; Newton et al. 2002; Pletnikov et al. 2008). For example, inducible bitransgenic mice were developed to express a dominant-negative form of CREB; these mice demonstrated greater cocaine-induced lowering of brain reward thresholds, but did not exhibit an increase in brain reward thresholds with administration of a κ opioid antagonist (Dinieri et al. 2009; Newton et al. 2002). Further, by crossing mice expressing these mutant genes with a knockout mouse lacking the gene of interest, restricted patterns of expression can be achieved, producing lines with alternate patterns of expression. While the resulting expression pattern cannot be predetermined, it may produce a desirable effect in which gene function is limited to a subset of neurons of interest. For example, a transgenic mouse was created to express the β2 nAChR receptor subunit under the control of TetOp in mice lacking the endogenous gene (β2 knockout mice). While three lines were generated, each with a different expression pattern, King et al. (2003) found that the mutant line expressing β2 nAChR subunits specifically in the cortex and thalamus exhibited an impairment in the expression of passive avoidance behavior, thus elucidating a function of β2-containing nAChRs in these brain regions. However, in addition to inducing expression in a subset of neurons of interest, ectopic expression may be induced in cells that do not normally express the endogenous gene. Depending on the inserted gene product, this may or may not introduce a confounding factor. For example, since the majority of the nAChR subunits only form functional receptor subtypes with the presence of other subunits, ectopic expression would not be an issue if the cell only expressed the inserted β2 nAChR subunit and no other nAChR subunit (King et al. 2003). Finally, as noted above with conventional knockout, the phenotype observed with genetic modification is in the context of the organism's genetic profile. As such, behavioral phenotypes may be differentially expressed based on the mouse's background strain (Hummel et al. 2004; Morice et al. 2004).

As opposed to creating multiple transgenic lines exhibiting variable patterns of gene expression and then selecting a line based on the expression pattern of interest, combining the inducible system with viral-mediated tTA expression may be preferred for cell-type specific inducible modification of gene activation. Nakanishi and colleagues generated a reversible neurotransmission blocking (RNB) tetracycline-responsive element to control the expression of tetanus toxin light chain in a doxycycline-dependent manner (Yamamoto et al. 2003). Tetanus toxin expression causes the cleavage of VAMP2, thereby eliminating synaptic neurotransmitter release. Inhibition of neurotransmitter release was achieved in striatal pathways projecting either to the substantia nigra or globus pallidus by injecting an adenovirus containing tTA with a substance P or enkephalin promoter, respectively, into the striatum (Hikida et al. 2010). Thereafter, reinstatement of VAMP2 expression and neurotransmitter release could be accomplished with doxycycline administration to terminate tetanus toxin gene activation. By utilizing these mice, the authors were able to demonstrate the differential roles of the striatonigral pathway in reward leaning and cocaine sensitization, and the striatopallidal pathway in aversive learning with the foot shock paradigm (Hikida et al. 2010).

Another strategy for inducible genetic modification is to use an inducible Cre system in which the recombinase remains in an inactive state until an inducing agent is administered

(Fig. 1E). The main advantage of this method is the ability to modify expression during a certain time period, such as during development, adulthood, or within the course of the development or expression of substance abuse-related behaviors. In these mice, the Cre recombinase is expressed in conjunction with a receptor normally localized to the cytoplasm (e.g., a hormone receptor) (Lambert 2009). When the inducing agent is administered, it binds to the receptor, leading to translocation of the Cre-receptor complex into the nucleus and subsequent removal of the floxed gene. Withholding the inducing agent permits restoration of gene expression. For example, the epigenetic regulator methyl-CpG-binding protein 2 (MeCP2) is essential for brain development, so McGraw et al. (2011) sought to modify gene expression during adulthood by utilizing a mouse with a floxed *Mecp2* gene and a tamoxifen-inducible Cre bound to the estrogen receptor allele ($Mecp2^{CreER}$). Tamoxifen was administered intraperitoneally for 20 days to sufficiently reduce MeCP2 levels, which resulted in neuronal dysfunction and behavioral deficits (McGraw et al. 2011). Konopka et al. (2010) employed a similar strategy to alter the expression of Dicer-dependent microRNAs by inactivating the *Dicer1* gene. However, in their study, the tamoxifen-inducible Cre (CreERT2) was associated with a CamKIIα promoter to limit gene modification to forebrain neurons (Konopka et al. 2010). Based on the role of MeCP2 and microRNAs in cocaine self-administration (Hollander et al. 2010; Im et al. 2010; Schaefer et al. 2010), these mouse lines may prove beneficial for additional investigations into mechanisms underlying cocaine reinforcement.

Optogenetic manipulations

With recent technological advances in optogenetics, genetically engineered mice have begun to prove invaluable for research approaches necessitating the modification of neuronal activity within a subset of neurons in a brain region of interest. By applying the optogenetic technique, insertion of opsin genes into mammalian cells permits manipulation of neuronal excitation or inhibition with the presentation of wavelength-specific light stimulation. Recent efforts to genetically modify the microbial opsin genes have led to a burgeoning array of optogenetic tools permitting more efficient manipulation of cellular function; these include the inhibitory halorhodopsin chloride pump, multiple versions of the excitatory cation channel channelrhodopsin (ChR2) that exhibit varying spectral and kinetic properties, and receptors for biochemical modulation of G proteins or cAMP [for review, see (Yizhar et al. 2011)]. Accordingly, a variety of viral vectors have been designed to selectively infect cells and permit expression of these light-mediated opsin receptors. Of most current interest for mouse geneticists is the ability to utilize vectors that selectively infect cells containing Cre, which can be accomplished with a Cre recombinase-dependent double-floxed inverted opsin gene in an adenoviral vector under a ER1α or CAG promoter (Yizhar et al. 2011). Through this approach, a specific subset of neurons may be selectively modulated at a desired time point. Within the past few years, numerous reports utilizing optogenetics have emerged with significant relevance to the field of drug addiction. Deisseroth and colleagues first demonstrated the development of a CPP with light-mediated phasic activation of dopaminergic neurons in the VTA by utilizing tyrosine hydroxylase::IRES-Cre transgenic mice (Tsai et al. 2009). Thereafter, studies have shown that activation of dopamine D1 neurons potentiate the rewarding effects of cocaine in the NAc, whereas activating D2 neurons or inhibiting cholinergic neurons in the NAc attenuates cocaine reward (Lobo et al. 2010; Witten et al. 2010). Further, reward seeking behavior can be supported by stimulating, or suppressed by inhibiting, amygdalar projection terminals in the NAc (Stuber et al. 2011), and activating dopaminergic neurons in the VTA enhances behavioral performance during a food-seeking task (Adamantidis et al. 2011). Optogenetics may also be combined with conditional knockout or inducible bitransgenic mouse lines (Katzel et al. 2011; Stuber et al. 2010). For example, Stuber et al. (2010) demonstrated that VGLUT2 is required for glutamate release from dopaminergic neurons projecting to the NAc by examining light-

mediated stimulation of exocytosis in mice lacking the VGLUT2 gene in dopaminergic neurons. Finally, inducible transgenic cells have been implanted into mice to permit light-controlled transcriptional activation (Ye et al. 2011); further studies with this type of mouse model may prove interesting.

Transgenic opsin mouse lines have also been employed to produce optical modulation of neuronal activity. Chuhma et al. (2011) crossed TetOp-ChR2 mice with CaM-KIIα-tTA mice to generate bitransgenic mice with selective expression of ChR2 in striatal medium spiny neurons. Although their tTA was originally designed to induce both excitatory and inhibitory control of neurons through expression of a bidirectional tetracycline promoter for both the ChR2 or halorhodopsin genes, only the ChR2 was functional. The authors demonstrated that medium spiny neurons form functional connections with other medium spiny neurons and cholinergic interneurons in the striatum, as well as with GABAergic neurons in the substantia nigra (Chuhma et al. 2011). While opsin mouse lines do not require the production of viral vectors and stereotaxic injection, several factors may limit their application for experimental approaches. The mouse lines expressing opsin genes often exhibit a weaker level of expression than that found with viral-mediated infection, and early developmental patterns of gene expression may induce irreversible recombination at the opsin locus, leading to persistent activation at a later time period (Yizhar et al. 2011). Further, it may not be possible to selectively modulate a desired subset of neurons in a brain region of interest if axons from Cre-containing cells in other brain areas also project into the brain region. For example, Ren et al. (2011) used a ChAT-ChR2-EYFP BAC transgenic mouse line to examine light-induced neurotransmitter release at MHb-IPN synapses. However, since ChAT is expressed both on projections into the IPN, as well as in local IPN neurons, ChR2 would likely be localized to both presynaptic and postsynaptic membranes within the IPN. Although the authors employed several pharmacological agents to modify neuronal activity in the IPN during light-mediated stimulation (Ren et al. 2011), which implicated presynaptic neurotransmitter release onto postsynaptic neurons, inward currents could not be completely abolished, potentially attributable to activation of ChR2 on the postsynaptic membrane.

Phenotype-selected mice

In addition to genetic modification, research efforts have sought to sort and/or preferentially bred mice based on certain behavioral phenotypes. For example, Nestler and colleagues examined behavioral responses to social defeat stress and subsequently grouped mice into susceptible and resilient populations (Vialou et al. 2010). Using this strategy, the authors established that the transcription factor ΔFosB differentially mediates the activation of the GluR2 and SC1 genes with behavioral resilience to stress, and mice overexpressing ΔFosB further validated the involvement of ΔFosB with SC1 gene modulation (Vialou et al. 2010). Further, selective breeding has been utilized to produce mice exhibiting differential withdrawal responses following chronic alcohol consumption (Kosobud and Crabbe 1986), and differences were found in gene expression during withdrawal between the seizure-resistant and seizure-prone mice (Hashimoto et al. 2011). Since genetic risk factors may underlie human susceptibility to alcohol's effects (Wetterling et al. 1999), this and further studies may lead to identification of important genetic mechanisms that contribute to human alcohol consumption.

Conclusions

Technological advances in the genetic engineering of mice have provided researchers with valuable mouse model systems for functional genomic investigations. In addition to the more conventional knockout and knockin strategies, the generation of conditional and

inducible methods has permitted neuron-specific regulation of gene expression within select brain regions. Advances in optogenetic tools have also led to more discrete manipulation of cellular function during behavior. Additionally, mouse models hold great promise for translational purposes with regards to target identification and validation of therapeutic mechanism of action and function, in addition to investigating the neuromechanisms involved in drug reinforcement and reward. In this respect, if a novel therapeutic is developed, the drug may be tested in knockout mice lacking the protein of action. If a drug-induced effect is displayed, one may conclude that the compound exerts off-target effects. Similarly, reporter mice may be used to verify the drug's effects or mechanism of action within specific neuronal populations (Berton et al. 2006; Boer et al. 2010). Another potential use of the mouse model for translation is with drug metabolism studies. Recently, Chen et al. (2011) developed a human ectopic artificial liver for transplantation into mice and demonstrated similar function relative to human drug metabolism and drug interactions. This represents a significant advance over prior studies that have relied on organs from cadavers which may be confounded based on donor characteristics (e.g., age, ethnicity, drug use history, postmortem time, etc.) (Sacca et al. 2010). These mice may be used to examine the metabolism of drugs of abuse and also to validate novel therapeutics for the treatment of substance abuse disorders. In summary, recent advances in the development of transgenic mouse models affords an excellent opportunity for novel investigations into the biological mechanisms underlying substance use and abuse. Data derived from human GWAS studies may be validated and further investigated with the mouse models, and conversely, findings attained from genetic manipulation of the mouse genome will likely lead to advances in our understanding of the human condition. These efforts may then merge to permit the development of novel therapeutics to prevent and treat substance addiction in humans.

Acknowledgments

Supported by the National Institute on Drug Abuse (DA020686 to PJK; DA026693 to CDF).

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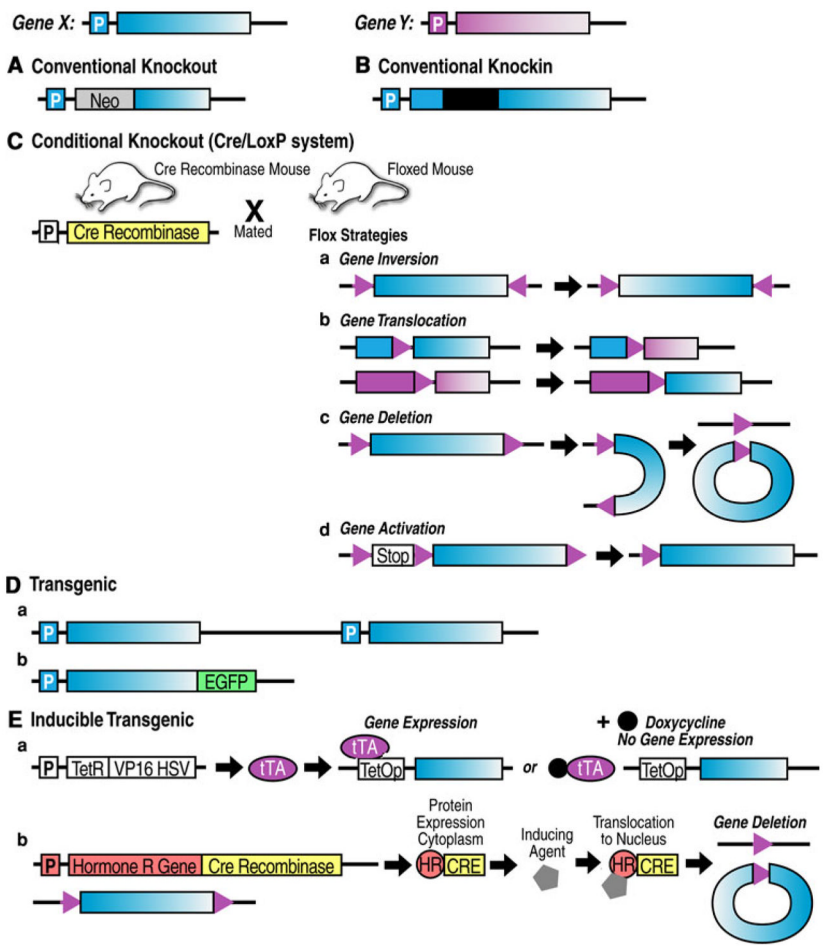

Fig. 1.

Genetically engineered mice may be generated with varying types of genetic modifications. The following examples provide an illustration of commonly employed genetic mutations for genetically engineered mouse models. **A** A conventional knockout mouse can be generated by inserting a Neo cassette into the gene to create a null mutation. **B** A conventional knockin may be produced by inserting the gene into the coding sequence of an endogenous site or by changing an amino acid sequence. **C** Conditional knockout models often employ the Cre/LoxP system. A mouse with Cre recombinase under a promoter of interest (e.g., depending on cell-specific modification desired) is crossed with a mouse that contains loxP sites (*purple arrows*) integrated into the genome (*floxed mouse*). Depending on the orientation and location of the loxP sites, the gene may be differentially modified by Cre recombinase. *a* If the loxP sites are inserted in opposing orientation surrounding the gene segment, the gene will undergo inversion. *b* If the loxP sites are inserted on two different genes, gene translocation can be achieved. *c* If the loxP sites are oriented in the same direction surrounding the gene segment, the gene segment will be removed, resulting in one remaining loxP site where the gene segment was located. *d* If a floxed stop codon is inserted with a loxP site in the same orientation at the other end of the gene segment, gene activation can be prevented. The presence of Cre recombinase catalyzes gene activation, thus permitting the gene to only be expressed in Cre-containing cells. **D** Transgenic mice can be created by inserting a genetic sequence into the genome with the BAC technology. This permits the generation of (*a*) overexpressing transgenic mice, or (*b*) reporter mice that have a tag, such as EGFP, fused to the gene product. It should be noted that this often leads

to multiple insertion sites in the genome (*a*). **E** For inducible regulation with transgenic mice, the most commonly employed methods are illustrated. *a* In the TetOp-tTA method, a tetracycline inhibitable transcription factor, tTA, fused to the VP16 herpes simplex virus (HSV)-encoded transcriptional activator is inserted under the control of an endogenous promoter (e.g., NSE), while the TetOp promoter is fused to the gene of interest. Under baseline conditions, tTA attaches to TetOp, and gene expression is permitted. With the presence of doxycycline (e.g., in the drinking water), doxycycline binds to tTA, thus preventing tTA from binding to TetOp and subsequent gene expression. This process may be reversed by removal of doxycycline. *b* The Cre recombinase gene is fused to a hormone receptor gene, such as progesterone, and the gene of interest is flanked by loxP sites. Under baseline conditions, the hormone receptor-Cre protein complex is expressed in the cytoplasm. When an inducing agent is administered, such as tamoxifen, the complex translocates to the nucleus and Cre recombinase removes the gene at the loxP sites