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Behavioral and Genetic Evidence for GIRK Channels in the CNS: Role in Physiology, Pathophysiology, and Drug Addiction

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Abstract

G protein-coupled inwardly rectifying potassium (GIRK) channels are widely expressed throughout the brain and mediate the inhibitory effects of many neurotransmitters. As a result, these channels are important for normal CNS function and have also been implicated in Down syndrome, Parkinson's disease, psychiatric disorders, epilepsy, and drug addiction. Knockout mouse models have provided extensive insight into the significance of GIRK channels under these conditions. This review examines the behavioral and genetic evidence from animal models and genetic association studies in humans linking GIRK channels with CNS disorders. We further explore the possibility that subunit-selective modulators and other advanced research tools will be instrumental in establishing the role of individual GIRK subunits in drug addiction and other relevant CNS diseases and in potentially advancing treatment options for these disorders.

1. INTRODUCTION

G protein-coupled inwardly rectifying K^+ (GIRK) channels are a family of ion channels that are activated via ligand-stimulated G protein-coupled receptors (GPCRs). Following ligand stimulation, activated G protein subunits are released that directly interact with and open GIRK channels so that they become permeable to K^+ ions. The outward K^+ current hyperpolarizes neuronal membranes and decreases neuronal excitability. GIRK channels are activated by a large family of GPCRs (reviewed in chapters "Unifying Mechanism of Controlling Kir3 Channel Activity by G Proteins and Phosphoinositides" by Logothetis et al. and "The Roles of Gβγ and Gα in Gating and Regulation of GIRK Channels" by Dascal and Kahanovitch), including dopamine 2 (D_2), serotonin 1A (5-HT_{1A}), μ-, κ-, and δ-opioid, cannabinoid 1 (CB_1), and γ-aminobutyric acid type B ($GABA_B$) receptors.

There are four mammalian subunits (GIRK1–4) with overlapping but distinct expression patterns throughout the CNS that form heterotetrameric channels (Karschin, Dissmann, Stuhmer, & Karschin, 1996; Koyrakh et al., 2005). GIRK2 and GIRK4 subunits can also form functional homotetrameric channels (Koyrakh et al., 2005; Krapivinsky et al., 1995). GIRK1–3 are considered the predominant subunits in brain, while GIRK4 expression is more restricted (Perry et al., 2008; Wickman, Karschin, Karschin, Picciotto, & Clapham, 2000). GIRK2 appears to be an integral subunit of most neuronal GIRK channels (Cruz et

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al., 2004; Luscher, Jan, Stoffel, Malenka, & Nicoll, 1997; Slesinger, Stoffel, Jan, & Jan, 1997). The expression patterns of GIRK subunits vary in individual brain regions and even among subcellular compartments within individual neurons, ensuring discrete regional and cellular signaling (reviewed in chapter “Localization and Targeting of GIRK Channels in Mammalian Central Neurons” by Luján and Aguado). It is interesting that the unique subunit composition of GIRK channels in different neuronal populations may confer distinct functional properties (Jelacic, Kennedy, Wickman, & Clapham, 2000; Jelacic, Sims, & Clapham, 1999; Schoots et al., 1999) and drug sensitivities that mediate the rewarding effects of certain addictive drugs, such as γ-hydroxybutyrate (GHB) (Cruz et al., 2004; Labouebe et al., 2007).

GIRK channels have been implicated in both normal CNS functions and pathological states (Luscher & Slesinger, 2010). They control key neurological processes, such as neuronal plasticity and learning/memory, and are sensitive to different drugs of abuse (see chapters “GIRK Channel Plasticity and Implications for Drug Addiction” by de Velasco et al. and “GIRK Channels: A Potential Link Between Learning and Addiction” by Tipps and Buck), making them relevant targets to examine in behavioral studies of cognition and drug addiction. In this chapter, we examine behavioral evidence from mouse knockout models as well as genetic studies from animal models and humans that support a role for GIRK channels in different CNS processes. This review includes normal responses such as pain perception, motor control, and memory formation, as well as GIRK contributions to the pathophysiology of Parkinson’s disease, Down syndrome, psychiatric diseases, and epilepsy. We also review the evidence for alcohol- and other drug-dependent behaviors that are mediated by GIRK-dependent signaling. Finally, we explore how recent progress in GIRK channel structural modeling (see chapter “Structural Insights into GIRK Channel Function” by Glaaser and Slesinger) and the development of subunit-selective channel modulators (Kaufmann et al., 2013; Ramos-Hunter et al., 2013; Wen et al., 2013) may advance understanding of channel function and, consequently, improve treatment options for many CNS diseases. These and other new research approaches may contribute to the design of better therapeutics for the CNS disorders that are associated with GIRK-dependent signaling.

2. GIRK CHANNELS IN CNS DISORDERS

Gene knockout mouse models have provided valuable insight into the role of GIRK channels in normal and pathological processes (Luscher & Slesinger, 2010). A primary concern of knockout lines is whether compensatory changes in the expression of other genes occur as a result of global deletion of individual genes. For example, GIRK1 protein levels are also decreased in mice lacking the *Girk2* gene (Signorini, Liao, Duncan, Jan, & Stoffel, 1997), and the lack of either GIRK1 or GIRK2 is correlated with lower expression of the other, suggesting a specific assembly between these subunits (Koyrakh et al., 2005; Marker, Stoffel, & Wickman, 2004). Although off-target effects of genetic deletion may be a confounding factor in knockout animals, these models have nevertheless been instrumental in generating information about the physiological relevance of GIRK channels. In sections 2 and 3, we outline the behavioral and genetic evidence in mice (Tables 1 and 2) and humans (Table 3), highlighting roles for GIRK channels in a variety of CNS disorders.

2.1 Cognitive Deficits

Several lines of evidence indicate that GIRK channels play a crucial role in cognitive function. GIRK channels are expressed in brain regions associated with learning and memory, including the hippocampus, amygdala, and pre-frontal cortex (Ehrengruber et al., 1997; Hearing et al., 2013; Luscher et al., 1997) and modulate the development of synaptic plasticity (Chung et al., 2009). Furthermore, changes in GIRK signaling are associated with learning impairments in behavioral studies of mutant mice (Table 1). For example, *weaver* mice, which contain a single amino acid mutation in the pore of the GIRK2 subunit (Patil et al., 1995), exhibited long-term learning deficits in models of instrumental learning (Derenne et al., 2007). *Girk4*$^{-/-}$ mice showed impaired performance in the Morris water-maze test but did not differ from wild-type mice in the passive avoidance test, indicating some impairment in spatial learning and memory but not aversive learning (Wickman et al., 2000).

Modulators of GIRK channel function may also be important in synaptic plasticity and learning and memory. Regulators of G-protein signaling (RGS) proteins accelerate GTPase activity of the Gα subunit, thereby negatively modulating GPCR–GIRK activation (reviewed in chapter “RGS Redundancy and Implications in GPCR–GIRK Signaling” by Doupnik). The R7 family of RGS proteins has prominent roles in motor control, nociception, and reward-related behavior in mammals, similar to some of the known roles for GIRK channels (Anderson, Posokhova, & Martemyanov, 2009). RGS7 and its binding protein R7BP control $GABA_B$ receptor ($GABA_BR$)–GIRK signaling in hippocampal pyramidal neurons (Ostrovskaya et al., 2014). Deletion of the *Rgs7* gene or its binding protein in mice increased the sensitivity to baclofen activation and slowed GIRK current deactivation in these neurons. The enhanced $GABA_BR$–GIRK coupling sensitivity and slower deactivation kinetics decreased neuronal excitability and disrupted inhibitory forms of synaptic plasticity, likely contributing to the learning and memory deficits observed in *Rgs7*$^{-/-}$ mice (Ostrovskaya et al., 2014). These knockout mice showed deficits in context recognition after fear conditioning and impairments in different aspects of the Morris water-maze test. Both of these tests rely on hippocampal processing for memory formation, providing corroborating behavioral evidence that RGS proteins control $GABA_BR$–GIRK signaling and mediate hippocampal synaptic plasticity.

Gene duplication and overexpression of GIRK subunits can also cause cognitive impairments. GIRK channels are implicated in the pathology of Down syndrome, a congenital disorder caused by an extra maternal copy (trisomy) of human chromosome 21 that is characterized by learning disabilities, craniofacial abnormalities, and hypotonia, although the extent of these phenotypes varies among individuals (Wiseman, Alford, Tybulewicz, & Fisher, 2009). The Down syndrome critical region (DSCR) of chromosome 21 contains several genes, including *KCNJ6*, which encodes the GIRK2 subunit (Toyoda et al., 2002). Transpolygenic mice carrying extra copies of chromosome 21 fragments covering the DSCR showed cognitive disabilities in most tests of the Morris water-maze and in the altered context stage in fear-conditioning tests (Chabert et al., 2004). The presence of *KCNJ6* in this region and the overexpression of GIRK2 and enhanced $GABA_BR$-dependent signaling may contribute to some of the mental disabilities in Down syndrome, as reviewed in Cramer, Best, Stoffel, Siarey, and Galdzicki (2010).

Different mouse models of Down syndrome have been generated that carry either a partial or full segment duplication of the analogous mouse chromosome 16, and these trisomy mice exhibit cognitive deficits (Liu et al., 2011). A partial trisomy 16 mouse model (Ts1Cje) containing the DSCR showed impaired performance levels in several tests in the Morris water-maze (Sago et al., 1998). Another partial trisomy model containing a larger region of chromosome 16 (Ts65Dn) showed greater learning impairment in Morris water-maze tests compared to Ts1Cje mice (Reeves et al., 1995). Ts65Dn mice were also more active in open-field tests and less responsive to environmental cues compared to controls (Coussons-Read & Crnic, 1996) and demonstrated deficits in a fear-conditioning test of associative learning (Costa, Scott-McKean, & Stasko, 2008). Ts1Rhr mice, which are trisomic for a small subset of the genes in Ts65Dn, and Ts1Cje mice showed cognitive deficits in novel object recognition and T-maze tests (Belichenko et al., 2009). Although all of these mice carry an extra copy of *Girk2*, other overexpressed genes on chromosome 16 may also contribute to the cognitive deficits observed in these models (Roubertoux & Carlier, 2010). The development of a mouse model with trisomy for *Girk2* alone provided more direct evidence for this gene in Down syndrome. These mice exhibited impaired hippocampal-dependent contextual-fear recall, altered responses to rewards, decreased excitatory synaptic plasticity, and increased long-term synaptic depression (Cooper et al., 2012). Recent evidence suggests that Keppen–Lubinsky syndrome, a rare disease characterized by physical abnormalities, lipodystrophy, developmental delays, intellectual disabilities, and microcephaly, is caused by mutations in *KCNJ6* (Masotti et al., 2015). Together, these studies highlight the importance of GIRK channels in cognition, in particular a role of GIRK2 (Tables 1 and 3).

2.2 Pain

Analgesics can target different types of GPCRs (e.g., opioid, cannabinoid, and $GABA_B$ receptors), and GIRK channels are a common effector of many analgesic medications (Pan et al., 2008). GIRK channels were first associated with pain perception based on the reduced analgesia observed in *weaver* mice after opioid or ethanol administration (Ikeda et al., 2000; Kobayashi et al., 1999). However, the *weaver* mutant is not an ideal model because the mutation causes neuronal degeneration rather than a selective loss of GIRK2 function (Patil et al., 1995). Viable mice lacking the *Girk2* gene were generated that lacked postsynaptic responses to neurotransmitters known to act through $G_{i/o}$-linked GPCRs, but retained normal presynaptic signaling (Luscher et al., 1997; Signorini et al., 1997; Slesinger et al., 1997). These knockout mice provided an improved model for studying the specific role of GIRK2-containing channels in neurophysiology and behavior.

In male $Girk2^{-/-}$ mice, there was a marked reduction or elimination of the antinociceptive effects of several compounds, including baclofen, ethanol, and a cannabinoid receptor agonist in the hot plate response test; however, analgesia induced by the NMDA receptor antagonist, ketamine, was not affected (Blednov et al., 2003). Although some of the drugs used in this study do not directly couple to GIRK channels, the loss of GIRK2 subunits may indirectly decrease neuronal sensitivity to these analgesics. Deletion of *Girk2* also blocked the endogenous opioid-dependent component of stress-induced analgesia, whereas nonopioid stress-induced analgesia was not altered. Other behavioral work corroborated

these findings, showing that morphine- and clonidine-induced antinociception were reduced in male *Girk2*$^{-/-}$ mice (Mitrovic et al., 2003). Both studies provided evidence that GIRK2 contributes to sex differences in nociception and indicated that activation of GIRK2-containing channels underlies the analgesic effects of a diverse array of drugs (ethanol, opioids, nicotine, cannabinoids, as well as alpha adrenergic, muscarinic cholinergic, and $GABA_B$ receptor agonists), suggesting a common mechanism for analgesic action.

Girk2$^{-/-}$ and *Girk3*$^{-/-}$ (but not *Girk4*$^{-/-}$) mice showed increased sensitivity to pain and blunted analgesic responses to morphine, with *Girk2*$^{-/-}$ mice being the least sensitive to morphine analgesia (Marker et al., 2002). *Girk2/3*$^{-/-}$ mice demonstrated greater hyperalgesia compared to the single mutants and were similar to *Girk2*$^{-/-}$ mice in blunted responses to morphine analgesia (Marker et al., 2002). Hyperalgesia and decreased analgesic responses following spinal administration of high doses of morphine were observed in *Girk1*$^{-/-}$ or *Girk2*$^{-/-}$ mice (Marker et al., 2004). Similar results were observed after administration of the GIRK channel blocker tertiapin in wild-type mice (Marker et al., 2004). GIRK1 and GIRK2 subunits are enriched in the superficial dorsal horn of the spinal cord (Marker et al., 2004), a key relay station for nociceptive processing, providing further evidence that spinal GIRK channels composed of GIRK1/2 subunits modulate thermal nociception and analgesia induced by high doses of morphine. In agreement with this, *Girk1*$^{-/-}$ or *Girk2*$^{-/-}$ mice showed blunted responses to μ- and *δ*- but not κ-opioid receptor agonists, and tertiapin reduced the ability of the μ- and *δ*-receptor agonists to increase the latency for tail withdrawal in the immersion tail-flick test (Marker et al., 2005).

Quantitative trait locus (QTL) mapping also identified *Girk3* as a candidate gene contributing to mouse strain-dependent differences in the analgesic effects of multiple drug classes, and *Girk3*$^{-/-}$ mice showed decreased analgesic responses to morphine and a cannabinoid receptor agonist (Smith et al., 2008). In humans, single nucleotide polymorphisms (SNPs) in *KCNJ6* (GIRK2) were associated with an increased requirement for opioid analgesics following abdominal surgery (Nishizawa et al., 2014, 2009). An SNP in *KCNJ6* was also associated with increased opioid requirement for analgesia and increased substitution therapy with methadone in former heroin addicts (Lotsch et al., 2010). A comparison of genetic variation in *KCNJ3* and *KCNJ6* identified SNPs in *KCNJ6* that were associated with a pain-related phenotype in patients following total knee arthroplasty with postoperative opioid analgesics (Bruehl et al., 2013). These studies suggest that genetic variation in GIRK-dependent signaling affects pain outcome in mice and humans (Tables 1 and 3). A review of GIRK signaling and therapeutic strategies in opioid-dependent analgesia is found in a recent review (Nagi & Pineyro, 2014).

2.3 Motor Control

Motor control is a dopamine-dependent behavior involving the nigrostriatal pathway. The dopamine neurons of the substantia nigra (SN), a key component of this pathway, express high levels of GIRK2-containing channels (Koyrakh et al., 2005; Reyes et al., 2012), suggesting that GIRK-mediated inhibition may contribute to motor activity. Increased motor activity was observed in both *weaver* (Schmidt et al., 1982) and *Girk2*$^{-/-}$ mice (Blednov et al., 2001a), supporting a role for GIRK2-containing channels in motor control (Table 1).

Girk2$^{-/-}$ mice showed transient hyperactivity and slower habituation in an open-field test and increased spontaneous locomotor activity during the dark phase in their home cages (Blednov et al., 2002). Motor activity increased after habituation and was inhibited by the dopamine D_1 receptor antagonist SCH 23390 and increased by the D_1 partial agonist SKF 38393. SCH 23390 also inhibited basal activity levels in knockout and wild-type mice. These results suggested that D_1 receptor signaling is enhanced in *Girk2*$^{-/-}$ mice in a stressful environment, resulting in transient hyperactive behavior.

Other work showed that both *Girk1*$^{-/-}$ and *Girk2*$^{-/-}$ mice displayed increased motor activity, delayed habituation to an open field, and resistance to baclofen-induced ataxia in the rotarod test (Pravetoni & Wickman, 2008), but *Girk3*$^{-/-}$ and *Girk4*$^{-/-}$ mice did not differ from wild-type in locomotor activity (Pravetoni & Wickman, 2008; Wickman et al., 2000). In contrast, ML297 (a potent small molecule agonist of GIRK1-containing channels) suppressed motor activity in wild-type C57BL/6J mice in an open-field test at the highest dose tested (Wydeven et al., 2014).

Further evidence for a role of GIRK2 in motor control comes from *weaver* mice, which have been used as a model of Parkinson's disease (Table 1). Parkinson's disease produces progressive degeneration of dopamine neurons in the SN pars compacta, resulting in loss of motor coordination and some cognitive impairment (Rodriguez-Oroz et al., 2009). The GIRK2 mutation in *weaver* mice makes the channels nonselective for cations and less sensitive to $G_{\beta\gamma}$ (Kofuji et al., 1996; Navarro et al., 1996; Slesinger et al., 1996), producing constitutively active GIRK2 channels that may be responsible for the neuronal degeneration in dopaminergic neurons in the SN pars compacta and cerebellar granular neurons that are observed in these mice (Harkins & Fox, 2002; Schmidt et al., 1982; Smeyne & Goldowitz, 1989). The progressive dopaminergic degeneration causes gait instability, poor limb coordination, and tremors, similar to the phenotypes observed in Parkinson's disease and chronic drug addiction (Ebadi et al., 2005). However, the neurological changes in *weaver* mice do not completely mimic those of Parkinson's disease. Activation of GIRK2 channels and the resulting efflux of K^+ have also been linked to nerve growth factor-programmed cell death in dorsal root ganglion neurons, which is important in normal development of the nervous system (Coulson et al., 2008), indicating that GIRK2 subunits control both normal and pathological mechanisms of neuronal degeneration.

2.4 Psychiatric Disorders

2.4.1 Depression—Depression is the most common psychiatric disorder and a leading cause of disability worldwide (Saltiel & Silvershein, 2015). First-line treatment options for depression include selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors, and norepinephrine–dopamine reuptake inhibitors (Saltiel & Silvershein, 2015). Although monoaminergic pathways are the primary targets of antidepressants, altered GIRK signaling may also be involved in their therapeutic action, considering that GIRK channels are the main inhibitory effectors of 5-HT_{1A} receptors. Deletion of GIRK2 subunits in mice was associated with depression-resistant behaviors combined with a reduced behavioral response to the antidepressant citalopram (an SSRI) and reduced electrophysiological responses to 5-HT_{1A} receptor agonists, suggesting that GIRK channels

may be targets for treating depression by decreasing sensitivity of dorsal raphe neurons to serotonergic transmission (Llamosas et al., 2015) (Table 1). Furthermore, chronic administration of fluoxetine, an SSRI, restored anticipatory behavior in socially stressed rats in a rodent model of depression, and this response was partly mediated by the suppression of $GABA_BR$–GIRK signaling (Cornelisse et al., 2007). The effect of fluoxetine on GIRK currents was also present in control animals and was independent of the animal's depressed state. High concentrations of fluoxetine inhibited GIRK channels expressed in oocytes, whereas other SSRI antidepressants had little or no effect (Kobayashi, Washiyama, & Ikeda, 2004). The GIRK channel inhibitor tipepidine also acts as a novel antidepressive agent in the forced swimming test in rats, perhaps by enhancing dopaminergic or noradrenergic transmission (Kawaura et al., 2012). In humans, an epistatic interaction between *KCNJ6* (GIRK2) and CREB1 (cyclic adenosine 5′-phosphate (adenosine monophosphate)-response element binding protein) may influence rumination, a symptom of depression (Lazary et al., 2011).

A recent study showed that the rapid antidepressant effects of NMDA receptor antagonists cause $GABA_BR$–GIRK decoupling by increasing the stability of the adapter protein, 14-3-3η (Workman et al., 2015). Interestingly, 14-3-3η is implicated in other neurological diseases that are associated with GIRK channel function, including Parkinson's disease and schizophrenia (Foote & Zhou, 2012). Levels of $GABA_BRs$ and 14-3-3η decreased in the hippocampi of socially defeated rats in a model of depression (Workman et al., 2015). However, mice injected with NMDA antagonists (rapid antidepressants) showed increased $GABA_BR$ and adaptor protein levels and decreased GIRK2 levels in hippocampal synaptoneurosomes. The elimination of $GABA_BR$–GIRK signaling via 14-3-3η was required for the rapid antidepressant efficacy of ketamine, suggesting that inhibition of GIRK signaling is a potential mechanism for treating depression, a condition associated with other psychiatric disorders and addictive behaviors.

2.4.2 Anxiety—There are many different types of anxiety disorders, and these disorders may increase the risk for comorbid mood and substance use disorders (Kessler, Ruscio, Shear, & Wittchen, 2010). Studies using knockout mice provide evidence for GIRK subunits in anxiety-related behaviors. For example, *Girk2*$^{-/-}$ mice demonstrated reduced anxiety with signs of hyperactivity in the elevated plus-maze, light/dark box, and "canopy" anxiety tests (Blednov et al., 2001a). In the elevated plus-maze, *Girk2*$^{-/-}$ mice spent a higher percentage of time in the open arms and had a greater number of total entries. A short period of social isolation decreased anxiety and increased total activity as shown by an increased number of open arm entries, whereas behavior in wild-type mice was not substantially altered by social isolation. In the light/dark box test, *Girk2*$^{-/-}$ mice demonstrated increased locomotion and a greater number of vertical explorations (rearings) in the light area. In the "canopy" test, increased locomotion in the exposed area and a trend to decrease the number of stretch attend postures in the most secure canopy area was observed in these mutants. Subsequent work showed decreased anxiety in the elevated plus-maze test in *Girk2*$^{-/-}$, and to a lesser extent in *Girk1*$^{-/-}$, but not *Girk3*$^{-/-}$ mice (Pravetoni & Wickman, 2008) (Table 1). As previously mentioned, deletion of GIRK2 subunits in mice resulted in decreased depressive-

like behaviors (Llamosas et al., 2015), suggesting that GIRK2-containing channels may be relevant targets for treating both anxiety and depression.

Direct activation of GIRK1-containing channels by ML297 also reduced anxiety-related behavior in mice, without producing addictive or sedative effects (Wydeven et al., 2014). The anxiolytic effect of ML297 in the elevated plus-maze test was lost in *Girk1*$^{-/-}$ mice. Observing the same behavioral phenotype using null mutants or a GIRK agonist administered to wild-type mice could indicate that either too little or too much GIRK activity is anxiogenic. Studies of ML297, or other derivatives, may uncover a useful class of anxiolytic compounds with fewer side effects.

GIRK channels may also be involved in obsessive–compulsive disorder (OCD), an anxiety disorder characterized by obsessions or compulsions that cause distress or interfere with daily function (Decloedt & Stein, 2010). Although SSRIs are the first choice for treatment of OCD, approximately half of the patients with resistant OCD fail to respond to these drugs (Decloedt & Stein, 2010). In a mouse model of OCD that examined marble-burying behavior, tipepidine potently and dose-dependently reduced marble-burying behavior and was effective at doses that did not affect locomotor activity (Honda, Kawaura, Soeda, Shirasaki, & Takahama, 2011). Thus, inhibition of GIRK signaling, either using pharmacological channel inhibitors or genetic deletion, is associated with reduced anxiety-related behaviors and may represent a novel mechanism for the treatment of these disorders.

2.4.3 Schizophrenia—Schizophrenia is a mental disorder that can manifest as disorganized thoughts, hallucinations, or delusions, although symptoms vary dramatically between patients and even change over time in individual patients (Buckley, Miller, Lehrer, & Castle, 2009). The comorbidity of schizophrenia with other psychiatric disorders (e.g., anxiety, depression, and substance abuse) is well documented (Buckley et al., 2009). A genomewide association study (GWAS) of schizophrenia in a Japanese population identified an SNP in *KCNJ3* (GIRK1) (Yamada et al., 2011), and a subsequent GWAS of *KCNJ3* in a Chinese population identified nine SNPs that were associated with schizophrenia (Yamada et al., 2012). The initial SNP marker in the Japanese population also showed significant association in the Chinese population. Furthermore, analysis of transcript levels in the dorsolateral prefrontal cortex from postmortem brains of patients with schizophrenia or bipolar disorder revealed lower expression of *KCNJ3* compared to control subjects (Yamada et al., 2012). Thus, *KCNJ3* may represent a susceptibility gene for schizophrenia in Asian populations (Table 3).

2.4.4 Attention Deficit Hyperactivity Disorder—Attention deficit hyperactivity disorder (ADHD) is a prevalent neurodevelopmental psychiatric disorder characterized by impulsivity, inattention, and/or hyperactivity (Wilens & Spencer, 2010). ADHD affects cognitive development and can have long-lasting effects on academic success and social relationships. The neurobiological causes of its symptoms are unclear, but there is evidence for overlap between ADHD and schizophrenia, mood disorders, and substance abuse (Hamshere et al., 2013; Larsson et al., 2013; Wilens & Spencer, 2010), all of which appear to involve GIRK signaling. Tipepidine, which inhibits GIRK channel activity, is used clinically as a nonnarcotic antitussive and was recently evaluated for its ability to improve

ADHD symptoms in pediatric patients. A preliminary pilot study indicated that tipepidine was well tolerated and improved ADHD rating scale scores (Sasaki et al., 2014). This suggests that inhibition of GIRK-dependent signaling might offer treatment options for ADHD that are safer than the current use of psychostimulants, which have undesirable side effects. Collectively, studies using tipepidine provide preliminary evidence for its role in different psychiatric disorders, including depression, anxiety, and ADHD.

2.5 Epilepsy

Epilepsy is a neurological disorder characterized by recurring epileptic seizures of varying duration and severity, which have no immediate underlying cause (Chang & Lowenstein, 2003). Mouse models have provided behavioral evidence that GIRK channels may be relevant targets for the treatment of seizure disorders (Table 1). The mutant GIRK2 channels in *weaver* mice are not selective for K^+ and upon activation, depolarize rather than hyperpolarize neurons, thus increasing neuronal excitability, likely contributing to the development of sporadic seizures observed in these mice (Eisenberg & Messer, 1989). *Girk2*$^{-/-}$ mice also developed spontaneous seizures and showed increased sensitivity to pentylenetetrazole-induced convulsions (Signorini et al., 1997). *Girk3*$^{-/-}$ mice did not develop seizures, but *Girk2/3*$^{-/-}$ mice experienced spontaneous and lethal seizures (Torrecilla et al., 2002). Seizures in these knockout mice may be explained by the overall loss of GIRK function and the decreased neuronal inhibition, which in turn, increases neuronal excitability. In addition, spinal administration of tertiapin, which blocks GIRK channels, had proconvulsant effects (Mazarati et al., 2006). Conversely, ML297, a selective activator of GIRK1-containing channels, had antiseizure efficacy in rat models of epilepsy (Kaufmann et al., 2013). Specifically, ML297 delayed seizure onset in an electroshock model and prevented convulsions and death in a chemical model of epilepsy (Kaufmann et al., 2013).

3. GIRK CHANNELS IN ADDICTION

The brain circuitry underlying addiction and the rewarding properties of drugs of abuse involves the mesocorticolimbic dopamine system, which consists of the ventral tegmental area (VTA), medial prefrontal cortex (mPFC), and nucleus accumbens (NAc) (van Huijstee & Mansvelder, 2014). GIRK channels are expressed in these regions, and GIRK signaling is altered by exposure to different types of addictive drugs, indicating that these channels are a common effector of drugs of abuse and likely mediate the neuroadaptations believed to be important in the development and progression of addiction. The location, cellular specificity, and long-lasting GIRK-mediated neuroadaptations triggered by different drugs of abuse suggest that GIRK channels contribute to expression of drug-addictive behaviors, such as drug seeking, craving, and relapse. In the following sections, we review the behavioral and genetic evidence from mouse models (Table 2) and genetic studies in humans (Table 3) that link GIRK channels with different drugs of abuse.

3.1 Ethanol

Alcohol initially produces intoxication, anxiolysis, and a sense of reward, presumably through direct action on specific targets such as ion channels or signaling cascades (Howard,

Trudell, & Harris, 2014; Trudell, Messing, Mayfield, & Harris, 2014). After prolonged and repeated exposure, alcohol-induced changes in gene expression and synaptic function are thought to contribute to the development of altered behaviors such as tolerance, sensitization, and compulsive consumption, the hallmark of addiction (Gilpin & Koob, 2008).

GIRK channels are implicated in ethanol action as discussed in a recent review (Bodhinathan & Slesinger, 2014). $GABA_BR$–GIRK transmission in dopamine neurons of the VTA (Federici, Nistico, Giustizieri, Bernardi, & Mercuri, 2009) and $GABA_BR$–GIRK currents in cultured cerebellar granule cells were enhanced by ethanol (Lewohl et al., 1999). Ethanol also directly activated exogenously expressed GIRK channels at concentrations that are intoxicating in humans (Aryal, Dvir, Choe, & Slesinger, 2009; Kobayashi et al., 1999; Lewohl et al., 1999). GIRK2 is a prominent GIRK subunit in brain (and the VTA), and GIRK2-containing channels expressed in oocytes were more sensitive to ethanol than GIRK1/4 or GIRK4 channels (Lewohl et al., 1999).

The direct alcohol sensitivity of GIRK channels and their location in brain regions that are implicated in drug and natural reward circuitry indicate that they are relevant targets for alcohol action *in vivo*, and mouse models have been fundamental for deciphering their role in ethanol responses (Table 2). The first behavioral studies examining the role of GIRK channels in ethanol responses used *weaver* mice, which lacked acute ethanol-induced analgesia, but not the sedative, hypothermic, and locomotor-activating effects of ethanol (Kobayashi et al., 1999). Reduced ethanol analgesia was also observed in *Girk2*$^{-/-}$ mice (Blednov et al., 2003), and *Girk2*$^{-/-}$ mice displayed greater ethanol-stimulated activity in an open-field test (Blednov et al., 2001b). Although the withdrawal severity after acute administration of ethanol was reduced in these mice, there was no difference in withdrawal severity following a chronic ethanol diet when the amount of ethanol given to knockout mice was matched with the amount consumed by wild-type. There were also no genotype differences in ethanol-induced sleep time or acute functional tolerance.

The role of GIRK channels in ethanol reward has been investigated using two common models of addictive behavior: ethanol consumption and conditioned place preference. Animal models of voluntary ethanol administration are valuable for profiling behavioral and genetic determinants in human alcoholics, who exhibit excessive consumption as a hallmark of the disease (Hyman, Malenka, & Nestler, 2006). Animal models of conditioned place preference and conditioned taste aversion provide insight into the rewarding and aversive effects of ethanol, while models of withdrawal profile dependence and the symptoms that contribute to susceptibility for continued drug abuse (Chester & Cunningham, 2002; Green & Grahame, 2008; Metten et al., 1998). Ethanol consumption and preference did not differ in wild-type and *Girk2*$^{-/-}$ mice in the standard two-bottle choice test where the bottle positions were alternated daily to control for position preferences. However, when the ethanol bottles were always available in the preferred location, *Girk2*$^{-/-}$ mice consumed more ethanol compared to wild-type (Blednov et al., 2001b). In addition, *Girk2*$^{-/-}$ mice showed reduced conditioned taste aversion for 2.0 and 2.5 g/kg ethanol (Hill et al., 2003). Unlike wild-type mice, *Girk2*$^{-/-}$ mice failed to develop a conditioned place preference for ethanol (Hill et al., 2003). Preliminary evidence reported by Tipps and Buck in chapter

"GIRK Channels: A Potential Link Between Learning and Addiction" indicates that *Girk3*$^{-/-}$ mice have increased preference for the ethanol-paired side compared to wild-type littermates. Thus, loss of GIRK2 or GIRK3 may produce opposite effects on ethanol-induced conditioned place preference, suggesting that the sensitivity to ethanol may depend upon the subunit composition of GIRK channels. Further evidence for subunit selectivity and ethanol sensitivity can be found in a recent study showing that deletion of GIRK3 increased limited access but not continuous access voluntary drinking and decreased acute withdrawal severity, but did not affect the metabolic, sedative, hypothermic, or ataxic effects of ethanol (Herman et al., 2015). Overexpression of GIRK3 in the VTA reversed the binge-drinking phenotype and reduced drinking in wild-type mice. Deletion of GIRK3 also decreased ethanol-induced excitation of VTA dopamine neurons and dopamine release in the NAc, providing additional evidence that GIRK3 is required for activation of the mesolimbic dopaminergic pathway by ethanol (Herman et al., 2015). These results point to a role for GIRK3 in the rewarding properties of ethanol and as a potential target for regulating binge-like drinking.

GIRK3 may also be required for other ethanol responses. For example, QTL mapping identified the *Girk3* gene in a region of mouse chromosome 1 that was associated with withdrawal from ethanol and other sedative– hypnotics (Kozell et al., 2009). DBA/2J and chromosome 1 congenic mice have a small QTL interval containing *Girk3* from the DBA/2J strain in a genetic C57BL/6J background and exhibit more severe withdrawal from ethanol and other drugs of abuse than C57BL/6J mice. *Girk3* expression in the brain is greater in these mice compared with C57BL/6J mice (Kozell et al., 2009). Furthermore, *Girk3*$^{-/-}$ mice demonstrate less severe withdrawal from ethanol than their wild-type littermates (Herman et al., 2015; Kozell et al., 2009). Interestingly, the region of chromosome 1 containing *Girk3* also contains QTLs for ethanol drinking (Tarantino, McClearn, Rodriguez, & Plomin, 1998), ethanol-conditioned aversion (Risinger & Cunningham, 1998), and acute sensitivity to ethanol (Crabbe, Belknap, Mitchell, & Crawshaw, 1994; Demarest, McCaughran, Mahjubi, Cipp, & Hitzemann, 1999), suggesting that this and other nearby genes may be involved in several ethanol-related behaviors.

A multivariate analysis of alcohol phenotypes in 37 different mouse mutant lines and their wild-type controls revealed that *Girk2* is part of a gene cluster associated with taste, and this cluster is driven by decreased ethanol and saccharin consumption (Blednov, Mayfield, Belknap, & Harris, 2012). The *Girk2* mutation was tested on two different backgrounds (B6×129, B6N6), which resulted in its placement in different gene clusters, suggesting that the genetic background also plays an important role in the function of this gene in alcohol-related phenotypes.

Human genetic studies have provided additional support for GIRK channels in alcohol-dependent phenotypes (Table 3). The Collaborative Study on the Genetics of Alcoholism (COGA) examined event-related oscillations (EROs) in electroencephalogram recordings, which signify cognitive processes during normal and pathological brain function (Basar, Basar-Eroglu, Karakas, & Schurmann, 2001). These brain oscillations are stable, highly heritable (van Beijsterveldt, Molenaar, de Geus, & Boomsma, 1996) and are shared between alcohol dependence and related disorders (Porjesz et al., 2005). A COGA family-based

GWAS was performed for a specific ERO phenotype using SNPs genotyped in families affected by alcohol use disorder. The GWAS identified several SNPs in *KCNJ6* (GIRK2) that may account for the ERO phenotype (Kang et al., 2012). SNPs in the promoter region of *KCNJ6* were also associated with alcohol dependence in adults and hazardous drinking behavior in adolescents who were exposed to early life stress (Clarke et al., 2011). Overall, genetic variations, subunit composition, and cell specificity can all be critical determinants of ethanol action on GIRK channel function and behavioral responses.

3.2 Sedative/Hypnotics

Sedative–hypnotic drugs depress CNS function and are used to reduce anxiety or induce sleep. Although alcohol, opioids, and GHB fall into this general category, barbiturates and benzodiazepines are considered the two major classes of sedative–hypnotics. A QTL associated with pentobarbital withdrawal was mapped to a region containing 15 genes of mouse chromosome 1, and *Girk3* was identified as a particularly promising candidate (Kozell et al., 2009). Less severe pentobarbital withdrawal was associated with lower *Girk3* mRNA expression, suggesting that a *Girk3* null mutation would decrease pentobarbital withdrawal compared to that of wild-type mice. As observed for ethanol, *Girk3*$^{-/-}$ mice experienced less severe withdrawal from pentobarbital and zolpidem (Table 2), providing evidence for GIRK3-containing channels in acute withdrawal from barbiturates and benzodiazepines (Kozell et al., 2009). *Girk3*$^{-/-}$ and wild-type mice did not differ in pentobarbital-induced sedation and hypothermia, suggesting that *Girk3* mediates a subset of sedative–hypnotic effects.

3.3 Psychostimulants

Psychostimulants, such as cocaine and amphetamine, can produce a continuum of behavioral and cognitive effects, with low doses producing beneficial cognitive effects and high doses producing addiction and psychosis (Wood, Sage, Shuman, & Anagnostaras, 2014). Behavioral studies in mice indicate that GIRK signaling may be involved in the early stages of addiction to cocaine (Table 2). *Girk2*$^{-/-}$ mice demonstrated enhanced locomotor responses to cocaine (Arora et al., 2010). In addition, mice given lentiviral RNAi infusions to suppress GIRK1 and GIRK2 expression in the mPFC showed elevated motor activity in response to an initial injection of cocaine (Hearing et al., 2013), suggesting that persistent suppression of GIRK signaling can presensitize mice to the motor-stimulatory effect of cocaine. Effects on drug seeking for psychostimulants were also observed in *Girk2*$^{-/-}$ and *Girk3*$^{-/-}$ mice. These mice exhibited reduced intravenous self-administration of cocaine compared to wild-type counterparts (Morgan et al., 2003). Interestingly, *Girk2/3*$^{-/-}$ mice self-administered more cocaine than *Girk2*$^{-/-}$ or *Girk3*$^{-/-}$ mice, perhaps due to differing compensatory mechanisms in the single versus double subunit knockouts.

Sorting Nexin 27 (SNX27) regulates GIRK channel trafficking and its expression is upregulated by cocaine and methamphetamine (Kajii et al., 2003). Mice lacking SNX27 showed a specific reduction in $GABA_BR$–GIRK currents in VTA dopamine neurons and were hypersensitive to the locomotor-stimulating effects of cocaine (Munoz & Slesinger, 2014). These effects were reversed by expression of GIRK2a, an SNX27-insensitive splice variant. Inhibition of $GABA_BR$–GIRK signaling, and the resulting increased dopamine

neuron excitability in the VTA, may be a cellular mechanism promoting addiction to psychostimulants.

3.4 Opioids

GIRK channels are coupled to μ-, κ-, and δ-opioid receptors (Nagi & Pineyro, 2014), and as discussed earlier, animal and human studies provide corroborating evidence that GIRK channels mediate opioid analgesia (Tables 2 and 3). In addition, GIRK signaling is important for the motor-stimulating effects of morphine. GIRK2/3 channels in VTA dopamine neurons were required for the motor-stimulatory effect of systemic morphine, whereas GIRK1/2 channels in VTA GABA neurons were not involved (Kotecki et al., 2015). Thus, GIRK channels appear to regulate opioid-induced motor activity in a cell- and subunit-dependent manner. This work illustrates the utility of selective ablation of GIRK subunits in individual neurons to decipher cellular- and subunit-specific GIRK signaling. GIRK2/3 channels in VTA dopamine neurons are also important in mediating the motor-stimulatory effects of cocaine (Munoz & Slesinger, 2014) and may be a common target for other drugs of abuse (Cruz et al., 2004; Herman et al., 2015; Labouebe et al., 2007).

GIRK channels are also implicated in dependence after chronic exposure to morphine (Tables 2 and 3). For example, morphine withdrawal symptoms were greatly reduced in mice lacking *Girk2/3*$^{-/-}$ (Cruz et al., 2008). Electrophysiological responses in brain slices from these mice lacked the increased spontaneous firing that is associated with morphine withdrawal, and postsynaptic GIRK currents were abolished. In humans, an SNP in *KCNJ6* (GIRK2) was associated with increased opioid requirements for analgesia and a lack of opioid withdrawal symptoms (Lotsch et al., 2010).

Interestingly, tolerance to repeated morphine administration may be associated with increased potency and sensitization of opioid receptors. Opioids have a biphasic effect on GIRK currents from periaqueductal gray neurons (which contribute to opioid antinociception and tolerance) in morphine-tolerant rats (Ingram, Macey, Fossum, & Morgan, 2008). GIRK currents were initially potentiated by met-enkephalin and inhibited by a μ-opioid antagonist in brain slices from morphine-pretreated rats, suggesting that repeated morphine exposure *in vivo* enhances agonist stimulation of μ-opioid receptors; however, peak GIRK currents in slices from morphine-tolerant rats exhibited greater desensitization. The altered μ-opioid–GIRK signaling may contribute to the development of opioid tolerance.

3.5 Nicotine

The first candidate gene study examining genetic risk variants of nicotine dependence identified a marker in *KCNJ6* (GIRK2) as one of the top signals (Saccone et al., 2007). We previously discussed the association between polymorphisms in *KCNJ6* and individual postoperative sensitivity to opioid analgesia in humans. One unique SNP (rs2835859) was also associated with susceptibility to nicotine dependence in a Japanese population (Nishizawa et al., 2014). Carriers of the C allele of this SNP were less sensitive to pain, required less opioid analgesics postoperatively, had higher susceptibility to nicotine dependence, and required a greater number of trials in order to stop smoking (Table 3).

Although there is no known connection between the nicotinic acetylcholine receptors activated by nicotine and GIRK channels, it is possible that these studies reflect a general shift in reward sensitivity mediated by differences in GIRK-dependent signaling.

3.6 GIRK Modulators and Other Drugs of Abuse

GIRK channels are implicated in ethanol and opiate withdrawal and cocaine seeking. It is interesting that RGS proteins are also associated with mediating effects of alcohol (Stewart et al., 2015), cocaine (Rahman et al., 2003), and morphine (Zachariou et al., 2003), although it is unknown whether these effects are related specifically to the modulatory influence of RGS proteins on GIRK-dependent signaling. In addition, GIRK channels may mediate some of the rewarding effects of tetrahydrocannabiol (the main component in marijuana), given that they are activated by endocannabinoids (Guo & Ikeda, 2004) and are implicated in cannabinoid-induced nociception and analgesia in knockout mice as previously discussed. GHB may prove to be another example in the list of addictive drugs that activate GIRK signaling. The different subunit composition of GIRK channels and $GABA_BR$–GIRK coupling efficiency in different types of VTA neurons may account for the cellular and behavioral effects of the $GABA_BR$ agonists, GHB (abused drug) and baclofen (anticraving drug) (Cruz et al., 2004; Labouebe et al., 2007). Unique neuronal populations and functional sensitivities may thus confer distinct regional and cellular control of GIRK channel function in brain. Based on their overall sensitivity (cellular, behavioral, genetic) to different types of drugs of abuse, GIRK channels likely constitute a common target in the addictive process with the potential to affect treatment outcome.

4. THERAPEUTIC POTENTIAL OF GIRK CHANNEL MODULATORS

The suggested roles for GIRK channels in CNS diseases are heavily based on studies using mouse knockout models. Although animal models have greatly advanced understanding of GIRK channel function in normal and disease states, extending studies of the genotypes and related phenotypes to humans are a necessary bridge. Behavioral evaluation of drug targets in animals, combined with analysis of genetic variants in humans, may provide an effective strategy for advancing therapeutics for drug dependence and other polygenic diseases. For example, a human genetic link between certain peroxisome proliferator-activated receptors (PPARs) and alcohol-related phenotypes corroborated studies in mice showing that specific PPAR agonists reduced ethanol consumption (Blednov et al., 2015). Combining both animal and human data to systematically evaluate and nominate specific GIRK subunits may be a beneficial approach for determining the significance of these channels in disease and their potential to affect treatment outcome.

Currently, effective pharmacotherapies for drug addiction disorders are lacking. Disulfiram, naltrexone (opioid antagonist), and acamprosate are FDA-approved for treating alcohol addiction, but have limited efficacy and are not routinely prescribed as therapeutics (Zindel & Kranzler, 2014). As discussed in this chapter, the $GABA_BR$–GIRK signaling pathway is involved in alcohol, cocaine, and GHB responses in animal studies. Interestingly, the $GABA_B$ agonist baclofen has been approved to treat alcohol addiction in France and is under clinical trials in the United States (Addolorato et al., 2011). A review of potential therapeutics for drug use disorders highlights the beneficial uses of baclofen and other

$GABA_BR$ modulators (Addolorato, Leggio, Hopf, Diana, & Bonci, 2012). Baclofen shows promise in managing alcohol-withdrawal symptoms, reducing alcohol craving, and promoting alcohol abstinence in preclinical animal models and human alcoholics. $GABA_BR$ agonists and positive allosteric modulators may also be effective for combating addiction to other drugs of abuse, including cocaine, methamphetamine, nicotine, and opioids (Phillips & Reed, 2014). In humans, baclofen reduced subliminal cue-induced mesolimbic activation in cocaine-dependent individuals, suggesting that it may be promising in preventing relapse (Young et al., 2014). Baclofen was also noted in a single case report as a potential treatment for GHB withdrawal (LeTourneau, Hagg, & Smith, 2008).

In addition to a role in mediating the effects of drugs of abuse, we discussed how $GABA_BR$–GIRK signaling is involved in mood, memory, and nociception. This suggests that there are other therapeutic applications for novel $GABA_BR$ modulators, as well as modulators of other GPCRs with GIRK channel effectors. For example, the development of novel opioid analgesics has the potential to improve chronic pain management while reducing side effects. An ideal opioid ligand might be one with selectivity for δ-opioid receptors that mediates its analgesic effects primarily via GIRK channels without inducing cellular tolerance or other unwanted effects involving other pathways (Nagi & Pineyro, 2014).

Preliminary results indicate that the GIRK channel inhibitor tipepidine may be effective in treating ADHD in children (Sasaki, Hashimoto, Tachibana, Kurata, Okawada, et al., 2014), and preclinical and preliminary clinical studies suggest that it may have uses in other psychiatric disorders, such as anxiety and depression (Honda et al., 2011; Kawaura et al., 2012; Sasaki et al., 2014). In addition, new classes of potent, subunit-selective GIRK channel compounds have been identified that could enable pharmacological targeting of particular brain regions and behaviors. For example, ML297 activates recombinant neuronal GIRK channels containing the GIRK1 subunit and decreases anxiety-related behavior without sedative or overt addictive (rewarding) effects (Wydeven et al., 2014). ML297, or another subunit-selective derivative, may provide better therapeutics for seizure and/or anxiety disorders and also help decipher the contribution of GIRK channels in other diseases. Ideally, new classes of potent compounds designed for therapeutic applications in CNS disease will offer both subtype selectivity and an enhanced ability to penetrate the blood brain barrier. These criteria would advance the possibility of finding specific modulators that can alter access to alcohol and other drug sites on GIRK subunits in brain. Inhibiting access to binding pockets in channel proteins that are targeted by drugs of abuse, without altering channel gating, offers a selective mechanism for treating drug abuse with decreased side effects. Identification of small selective molecules, along with advances in X-ray crystallography of channel structure (discussed below), are promising tools for drug design and manipulation of discrete sites of channel action. Targeted molecules, acting on GIRK or other channels that are modulated by drugs of abuse, make the goal of therapeutics with decreased side effects an exciting prospect for addiction research.

Furthermore, other technologies (knock-in animal models, conditional and cell-specific knockouts, light activation of GPCRs, and chemogenetic applications for GPCRs, such as Designer Receptors Exclusively Activated by Designer Drugs or DREADDs) (Urban &

Roth, 2015) provide discriminating tools for the future and allow more targeted approaches compared to studies of global null mutants. Human genetic association studies are also needed to determine candidate genes and more accurately assess the role of GIRK channels in CNS disorders. X-ray crystal structures have shown ethanol bound to ion channels and provide evidence for alcohol-binding cavities in GIRK (Aryal et al., 2009) and other channel proteins (Howard et al., 2014). The rapid progress being made in crystal structures will be key for modeling the interactions of drugs with GIRK subunits and diagramming the rules of engagement (see chapter “Structural Insights into GIRK Channel Function” by Glaaser and Slesinger). The physical, chemical, and modulatory properties of drug-binding pockets may reveal mechanisms or smart molecules that can displace and inhibit drug action on GIRK channels (Bodhinathan & Slesinger, 2014). Applying structural data with the aforementioned approaches can help connect molecular models to function and behavior and enhance translational research. However, these goals and ideals are tempered by the fact that full activation or inhibition of GIRK function, even in discrete areas, could profoundly alter the balance of excitatory/inhibitory signaling and produce unwanted consequences.

5. CONCLUDING REMARKS AND FUTURE DIRECTIONS

Mouse knockout models have been invaluable for determining the roles of GIRK channels in many different CNS processes, but continued progress likely requires more combinatorial approaches to bridge animal and human studies, as well as the implementation of new tools. Collectively, the resources and approaches described above (i.e., subunit-selective channel modulators, conditional knockouts, crystallography, etc.) will be crucial for deciphering the role of GIRK channels in disease and in drug design for individual pathological conditions. Advances in these different areas of research will be quite significant in determining if GIRK channels are indeed potential targets for treating CNS disorders. Although GIRK channel function is altered in many CNS diseases, other protein targets and signaling mechanisms are also affected, and the specific role of GIRK channels and their modulators must be convincing before they can be considered for therapeutic benefit.

Biological systems, including the pathological processes operating in CNS disorders, function within a framework of inter-connected pathways. Ultimately, successful treatment of complex-trait disorders will depend on a systems-level approach to disease. While the study of individual genes is informative, using network-centric and systems biology approaches to identify inter-related gene networks and pathways that are more likely representative of the array of processes operating in CNS diseases are also warranted. Rapid advances in genomic and proteomic techniques can transform our ability to analyze complex disease processes and decode large data sets into more meaningful biological processes (Gorini, Harris, & Mayfield, 2014). Because drug addiction and other CNS diseases represent multifactorial processes with genetic and environmental determinants and neuroadaptations related to disease progression (Renthal & Nestler, 2008), moving beyond the significance of individual candidate genes to include the relevant gene and protein networks may better ascertain the role of GIRK channels and their associated biological systems in different stages of disease.

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Table 1

Mouse Girk Genes Implicated in CNS Disorders

Behavioral Phenotypes	GIRK Genotypes	References
Learning/memory deficits	*Girk2* (*weaver*)	Derenne, Arsenault, Austin, and Weatherly (2007)
	Girk4 (−/−)	Wickman et al. (2000)
	Rgs7 (−/−)[a]	Ostrovskaya et al. (2014)
Down syndrome model	*Girk2* (triploid)	Cooper et al. (2012)
Reduced analgesia	*Girk1* (−/−)	Marker et al. (2004); Marker, Lujan, Loh, and Wickman (2005)
	Girk2 (*weaver*	Ikeda, Kobayashi, Kumanishi, Niki, and Yano (2000); Kobayashi et al. (1999)
	Girk2 (−/−)	Blednov, Stoffel, Alva, and Harris (2003); Cruz et al. (2008); Marker, Cintora, Roman, Stoffel, and Wickman (2002); Marker et al. (2004, 2005); Mitrovic et al. (2003)
	Girk3 (−/−)	Marker et al. (2002); Smith et al. (2008)
	Girk2/3 (−/−)	Cruz et al. (2008); Marker et al. (2002)
Increased motor activity	*Girk1* (−/−)	Pravetoni and Wickman (2008)
	Girk2 (*weaver*)	Schmidt et al. (1982); Harkins and Fox (2002)
	Girk2 (−/−)	Blednov, Stoffel, Chang, and Harris (2001a); Blednov et al. (2002)
Parkinson's symptoms	*Girk2* (*weaver*)	Caviness and Rakic (1978); Coscia and Fentress (1993); Derenne et al. (2007); Schmidt et al. (1982)
Increased seizures	*Girk2* (*weaver*)	Eisenberg and Messer (1989)
	Girk2 (−/−)	Signorini et al. (1997)
	Girk2/3 (−/−)	Torrecilla et al. (2002)
Reduced anxiety-like behaviors	*Girk1* (−/−)	Pravetoni and Wickman (2008)
	Girk2 (−/−)	Blednov et al. (2001a); Pravetoni and Wickman (2008)
Increased depressive-resistant behaviors	*Girk2* (−/−)	Llamosas, Bruzos-Cidón, Rodríguez, Ugedo, and Torrecilla (2015)

Girk genes and associated phenotypes from *weaver*, knockout (−/−), and triploid mouse models are shown.

[a] *Rgs7* (regulator of G-protein signaling) is not a GIRK gene, but accelerates G-protein inactivation and negatively modulates GIRK responses (Lujan, Marron Fernandez de Velasco, Aguado, & Wickman, 2014). Parkinson's disease-like behavioral phenotypes include cognitive impairments, hyper-reactivity, ataxia, poor limb coordination, and tremors.

Table 2

Role of Mouse Girk Genes in Mediating the Behavioral Effects of Drugs of Abuse

Drugs of Abuse	GIRK Genotypes	Behavioral Phenotypes	References
Ethanol	*Girk2* (*weaver*), *Girk2* (−/−)	↓ Analgesia	Kobayashi et al. (1999); Blednov et al. (2003)
	Girk2 (−/−)	↓ CTA/CPP	Hill, Alva, Blednov, and Cunningham (2003)
	Girk2 (−/−), *Girk3* (−/−)	↓ Acute withdrawal severity	Blednov, Stoffel, Chang, and Harris (2001b); Kozell, Walter, Milner, Wickman, and Buck (2009); Herman et al. (2015)
	Girk2 (−/−)	↑ Intake[a]	Blednov et al. (2001b)
	Girk3 (−/−)	↑ Binge-like drinking	Herman et al. (2015)
Pentobarbital Zolpidem	*Girk3* (−/−)	↓ Withdrawal	Kozell et al. (2009)
Cocaine	*Girk2* (−/−)	↑ Motor activity	Arora et al. (2010)
	Girk2 (−/−), *Girk3* (−/−)	↓ Self-administration	Morgan, Carroll, Loth, Stoffel, and Wickman (2003)
Opioids	*Girk2* (*weaver*)	↓ Morphine analgesia	Ikeda et al. (2000)
	Girk1 (−/−), *Girk2* (−/−)	↓ Opioid analgesia	Blednov et al. (2003); Marker et al. (2004,2005); Mitrovic et al. (2003)
	Girk2/3 (−/−), *Girk3* (−/−)	↓ Opioid analgesia	Cruz et al. (2008); Marker et al. (2002); Smith et al. (2008)
	Girk2 (−/−)	↑ Morphine-induced motor activity	Kotecki et al. (2015)
	Girk3 (−/−)	↓ Morphine-induced motor activity	Kotecki et al. (2015)
	Girk2/3 (−/−)	↓ Morphine withdrawal	Cruz et al. (2008)

The behavioral effects of drugs of abuse and the related *Girk* genes from *weaver* and knockout (−/−) mice are shown.

CTA, conditioned taste aversion; CPP, conditioned place preference.

[a]Ethanol intake only increased when the ethanol bottles were available in the preferred location, but not when the positions were alternated daily to control for side preferences. Kotecki et al. (2015) also showed that selective ablation of *Girk2* in VTA dopamine neurons increased morphine-induced motor activity, and the diminished activity in *Girk3* (−/−) mice was rescued by restoring GIRK3 expression in the VTA.

Table 3

Human KCNJ Genes Implicated in CNS Disorders

Behavioral Phenotypes	Genotypes	References
Down syndrome	*KCNJ6*	Toyoda et al. (2002)
Keppen–Lubinsky syndrome	*KCNJ6*	Masotti et al. (2015)
Schizophrenia	*KCNJ3* SNPs	Yamada et al. (2011, 2012)
Increased opioids required for analgesia	*KCNJ6* SNPs	Bruehl et al. (2013); Lotsch, Pruss, Veh, and Doehring (2010); Nishizawa et al. (2009, 2014)
Reduced opioid withdrawal	*KCNJ6* SNPs	Lotsch et al. (2010)
Nicotine dependence	*KCNJ6* SNPs	Nishizawa et al. (2014); Saccone et al. (2007)
Alcohol dependence (adults) Hazardous drinking (adolescents)	*KCNJ6* SNPs	Clarke et al. (2011)
EROs in alcohol dependence	*KCNJ6* SNPs	Kang et al. (2012)

The human *KCNJ* genes associated with CNS disorders and drugs of abuse are shown. *KCNJ3* and *KCNJ6* correspond to the mouse *Girk1* and *Girk2* genes, which encode the GIRK1 and GIRK2 subunits, respectively.

EROs, event-related oscillations; SNPs, single nucleotide polymorphisms.