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Emerging concepts for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease

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Abstract

G protein-gated inwardly rectifying potassium *(*GIRK) channels hyperpolarize neurons in response to the activation of many G-protein coupled receptors and thus control the excitability of neurons through GIRK-mediated self-inhibition, slow synaptic potentials and volume transmission. GIRK channel function and trafficking are highly dependent on their subunit composition. Pharmacological investigations of GIRK channels and studies in animal models suggest that GIRK activity has an important role in physiological responses, including pain perception and memory modulation. Moreover, abnormal GIRK function has been implicated in altering neuronal excitability and cell death that may be important in the pathophysiology of human diseases such as epilepsy, Down's syndrome, Parkinson's disease and drug addiction. GIRK channels may therefore prove to be a valuable new therapeutic target for treating these health problems.

Introduction

Much of the interplay of excitatory and inhibitory signals required for normal neuronal function occurs in the dendrites of neurons[1]. Detailed electron and light microscopic studies show that fast excitatory inputs are mediated by ionotropic glutamate receptors such as NMDARs and AMPARs that are located in the postsynaptic density at the head of the spine, whereas inhibitory synapses typically form on the soma and dendritic shafts[1]. The fast inhibitory signals are mediated by ionotropic $GABA_A$/glycine receptors. In addition to this fast inhibition, a slower inhibitory postsynaptic potential (sIPSC) exists that is mediated by G protein-activated inwardly rectifying potassium (GIRK or Kir3) channels that are located perisynaptically outside the postsynaptic density in the spine and also on the shaft[2]. This review discusses recent findings on the physiology, function and dysfunction of GIRK channels in the brain.

Physiology of GIRK channels

GIRK channels are members of a large family of inwardly rectifying potassium channels (Kir1 – Kir7). The term "inward rectification" refers to a change in slope of the current-voltage relationship at the reversal potential (i.e. the zero current level, which occurs at the equilibrium potential for K^+ - E_K). Therefore the outward current is smaller (but not zero)

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than the inward current (Figure 1A), due to occlusion of the central pore by intracellular Mg^{2+} and polyamines[3]. Under physiological conditions the resting membrane potential of a typical neuron is positive to E_K, and the small outward K^+ current through GIRK channels decreases the excitability of a neuron (Figure 1A, large arrow). Different types of neurotransmitters, such as acetylcholine, dopamine, opioids, serotonin, somatostatin, adenosine and GABA, activate these channels by stimulating their cognate G protein-coupled receptors (GPCRs), which in turn couple specifically to pertussis toxin (PTX) sensitive heterotrimeric G proteins that activate GIRK channels[4]. Whether the activated Gα subunit or Gβγ dimer directly opened GIRK channels was the focus of an animated debate in the 1980s (**see** Box 1). Now, a preponderance of evidence supports the conclusion that Gβγ dimers released from PTX-sensitive (Gαi/Gαo) G proteins bind directly to GIRK channels causing them to open[5-9] (Box 1).

Building neuronal GIRK channels

Mammals express four GIRK channel subunits (GIRK1-4 or Kir3.1-3.4). In the brain, GIRK1-GIRK3 subunits are common (Figure 1B), while GIRK4 expression is low and therefore does not significantly contribute to cerebral GIRK currents10. The three splice variants of GIRK2 that are expressed in the brain, differ in the length of the C-terminal domain: GIRK2c contains a PDZ-binding motif that is absent in GIRK2a and GIRK2b[11-14]. For clarification, GIRK2a (Kir3.2a) has been previously referred to as GIRK2[14] and GIRK2-115, while GIRK2c has been previously referred to as KATP-216, GIRK2A-114,[15] and BIR1[17]. GIRK1 splice variants exist in the brain but have not been investigated in detail[18] and no splice variants have been reported for GIRK3 and GIRK4. GIRK subunits assemble into tetrameric channels in both heterologous expression systems (e.g. *Xenopus* oocytes, HEK-293 cells) and native tissues3,19[-23]. Because GIRK1 and GIRK3 subunits are unable to form functional channels on the plasma membrane, they form heterotetrameric channels (GIRK1/3; GIRK2/3)[20,24,25] (Figure 1B). GIRK2, on the other hand, is unique because it can form homotetramers[11,22,23] and can assembles with other GIRK subunits to form heterotetramers, such as GIRK1/2 and GIRK2/3.

Biochemical and molecular genetic experiments indicate that the predominant form of GIRK channels in the brain is a heterotetramer of GIRK1 and GIRK2 subunits[19]. Whether asymmetric arrangements of GIRK channel heterotetramers, such as a GIRK1-GIRK1-GIRK2-GIRK3, exist in neurons is still not known. Studies with GIRK knockout mice demonstrate that GIRK2 plays a primary role in generating GIRK currents in neurons (Table 1); mice lacking GIRK2 channels exhibit little or no GIRK current in a number of brain regions (Figure 1D, Table 1), including the hippocampus, cerebellum, substantia nigra, locus coeruleus and VTA[26-32]. Interestingly, GIRK3 knockout mice are indistinguishable from wild-type controls in some behavioral tests[33] (e.g. open-field motor activity and motor-coordination) and have similar agonist-induced currents but exhibit less severe withdrawal from sedatives[34] and reduced cocaine self-administration35. These findings suggest GIRK3 may have a regulatory role for GIRK signaling in the brain.

Single-channel recordings reveal that the biophysical properties of heterotetrameric and homotetrameric channels differ. Whereas GIRK2 homotetrameric channels have short opening times (<0.5 ms mean open time), the heterotetrameric channels (e.g. GIRK1/GIRK2, GIRK1/GIRK3 or GIRK1/GIRK4) display longer opening times (~1-2 ms mean open times)[3,11,23,25,36]. The significance of different single channel kinetics is not clear but the direct association of G protein Gβγ subunits with GIRK channels might alter the frequency of long openings, giving rise to large receptor-activated currents[37,38].

Interestingly, the single-channel conductances for channels expressed in heterologous expression systems (38-40 pS) are fairly uniform compared to those observed in neurons, which reveal a variety of brain region-specific differences (see Table 2). In native tissues, the subunit composition is not known but single-cell RT-PCR data have recently provided information about possible types of heterotetramers (Table 2). Differences in conductance between native and heterologously expressed GIRK channels suggest that the subunit composition of native channels may not be symmetric (e.g. two GIRK1, one GIRK2 and one GIRK3) or that there are channels composed of currently unknown subunits. More detailed single channel and biochemical studies are needed to resolve these differences.

GIRK motifs: trafficking and binding partners

A number of intrinsic amino acid sequences have been identified that control the intracellular trafficking of GIRK channels. GIRK2 contains a strong ER export signal (acidic residues) as well as an internalization ("VL") motif[39], enabling this subunit to form homotetramers or heteroteramers. GIRK1, on the other hand, lacks an ER export signal and must associate with another GIRK subunit (e.g. GIRK2 or GIRK3) to express on the plasma membrane24،39.. Although GIRK3 cannot form functional homotetramers, it can coassemble with GIRK1 or GIRK2 to form functional heterotetramers11،20،23،25. Unique to GIRK3, however, is a lysosomal targeting sequence ("YWSI") that promotes degradation of GIRK channels[39]. Both GIRK2c and GIRK3 contain a class I PDZ binding motif, and interact directly with a novel PDZ-containing trafficking protein, sorting nexin 27 (SNX27)40. Association of SNX27 with GIRK3-containing channels leads to a reduction of GIRK signaling on the plasma membrane, most likely by promoting its internalization40. Whether GIRK2c and GIRK3 also interact with classical PDZ containing proteins, such as PSD95/SAP97, in neurons is unclear41،42. The interplay of these trafficking motifs suggests that GIRK2 plays a primary role in forming native GIRK currents while GIRK3 may regulate the availability of GIRK channels on the plasma membrane.

Though most neurons express GIRK1, 2 and 3, dopamine (DA) neurons of the SN and VTA do not express GIRK1-containing GIRKs. In SN dopamine neurons, for example, GIRK channels are comprised of GIRK2a/2c subunits[41]. In the VTA dopamine neurons, some GIRK channels are heterotetramers of GIRK2c/GIRK343. It is not well understood why DA neurons express a unique combination of GIRK subunits but GIRK2/3 channels are less sensitive to Gβγ subunits and exhibit a higher EC_{50} for coupling to $GABA_B$ receptors than GIRK1-containing channels20،[43]. This difference in sensitivity has been linked to important changes in the output of the drug reward pathway (see below).

Several modulators have been described that alter GIRK channel activity, including Na^+[44,45], ethanol[46-48] and phosphorylation by PKA[49,50] and PKC kinases[51-57]. Both Na^+ and ethanol appear to stimulate GIRK channels through a specific binding site on the channel (Figure 2, see below). PKC-dependent phosphorylation decreases while PKA-dependent phosphorylation enhances channel activity[49-57] (Figure 2C). In addition to these modulators, changes in the levels of the membrane phospholipid phosphatidylinositol 4,5 bisphosphate (PIP_2), can regulate the activity of GIRK channels58-[60]. Stimulation of GPCRs that couple to Gq G proteins stimulate phospholipase C (PLC) and deplete plasma membrane levels of PIP_2, leading to desensitizing GIRK currents 55،61،62 (but see ref63). Activation of PLC also leads to stimulation of PKC, enabling cross-talk of these two pathways (PIP_2 depletion and PKC phosphorylation) in the regulation of GIRK channels[55,56].

Molecular and Structural Insights into GIRK function

Although a high-resolution three-dimensional structure of a full-length GIRK channel is currently not available, structures of the cytoplasmic domains of GIRK1, GIRK2 and a G protein-insensitive inward rectifier (IRK1) have been solved[64-66]. Comparison of these structures highlights a conserved secondary structure for inwardly rectifying potassium channels, consisting of 14 beta strands and two alpha helices (Figure 2A,B). These cytoplasmic high resolution structures have revealed a new structure, the G loop formed by the βH-βI sheet[65], that is important in channel gating[65,67,68], and identified several amino acids involved in regulating inward rectification and K^+ binding[64,69-71]. A recent 3D structure of a full-length chimeric channel, containing the transmembrane domains and pore of a bacterial inward rectifier (KirBac1.3) channel, and the cytoplasmic N- and C-terminal domains of GIRK1 has been solved in two different conformations[72] revealing the relative position of the pore (selectivity filter), G-loop and M2 gates, and the cytoplasmic interaction sites (Figure 2A). Notably, these crystals revealed two different positions of the G loop, which have been tentatively ascribed to putative open and closed states of the channel[72]. Though more studies are needed, a structural view of GIRK channel gating is emerging that involves the N-terminal slide-helix[73], K^+ and polyamine binding sites[69,70,71,74], and movement of the M2 and G loop gates[65,69,71,72,73].

Having high-resolution structures of GIRK channels provides key information on the structural context of sites previously identified for G protein regulation. GIRK channels are predominantly closed at resting membrane potentials (agonist-independent, basal activity) and become activated upon stimulation of PTX-sensitive Gi/o G proteins (receptor-induced current). Both forms of activation involve binding of Gβγ directly to the GIRK channel[8,9,36,75]. Functional and biochemical studies have identified sequences in the N- and C-terminal domains of GIRK1-4 that are involved in both agonist-independent and receptor-induced Gβγ activation[9,75-82] (Figure 2A,B). In particular, a Leu (GIRK1-L333, GIRK2-L344, GIRK4-L339) in the C-terminal domain of GIRK channels (βL-βM sheet) appears to have a critical role in the Gβγ-dependent activation of GIRK channels[77,80,81]. Similarly, functional and biochemical studies have pinpointed Gα binding domains in the N- and C-terminal domains of GIRK channels[82-85,99].

Mapping these G protein-interaction sites on the high-resolution structures provides clues to possible mechanisms of Gβγ gating (Figure 2A,B). For example, many of the regions implicated in Gβγ activation fall on the external side of the cytoplasmic domains, along an interface formed by two adjacent subunits (Figure 2B). Originating from different subunits, sequences from the N-terminal domain and the βD-βE and βL-βM sheets in the C-terminal domain contribute to this interface. An interaction between the N- and C-terminal domains of two subunits has been shown to be important for assembly and gating of GIRK channels [86]. Recently, a hydrophobic pocket has been identified in this subunit interface and implicated in G protein-dependent activation of GIRK channels[46]. FRET (fluorescence resonance energy transfer, see Box 2) measurements further reveal that Gβγ activation elicits a structural change, possibly a rotation, in the GIRK N- and C-terminal domains[87]. Systematic mutagenesis studies have indicated that specific amino acids in the M2 transmembrane domain[88-90] and G-loop[65,67,68] form a barrier to ion conduction in the closed state; thus conformational changes triggered in the cytoplasmic domains must couple to the channel's gates to open the channel. For example, the binding of Gβγ subunits to the channel could induce a conformational change involving the βL-βM sheets (containing the Leu implicated in Gβγ activation), the N-terminal domain, and the slide-helix, which, in turn, is relayed to the M2 and G-loop gates near the membrane. High-resolution structures of Gβγ or Gαβγ complexed with full-length GIRK channels will be necessary to clarify our

understanding of the molecular and structural mechanism underlying Gβγ activation and to identify amino acids in the Gβγ and Gα subunits that interact with GIRKs.

High-resolution structures of inwardly rectifying K^+ channels have also enabled visualization of amino acids implicated in PIP_2 binding as well as possible gating mechanisms for ethanol and Na^+ modulation. Clusters of basic (positively charged) amino acids of the cytoplasmic domain58-60 are positioned in close proximity to the plasma membrane, where they are poised to interact directly with negatively changed membrane phospholipids and couple with the two gates (M2 and G loop) (Figure 2A). Recently, a hydrophobic pocket formed by the N-terminal domain, and the C-terminal βD-βE and βL-βM sheets has been identified as a site for ethanol activation of GIRK channels[46]. Bulky amino acid substitutions of a Leu located in the βD-βE sheet of the hydrophobic pocket decreases ethanol-dependent activation[46] (Figure 2B). A structural analysis of GIRK2 has also provided a possible mechanism for Na^+ dependent regulation of GIRK channels: Na^+ may promote PIP_2 binding to GIRK channels by breaking a hydrogen bond formed between an aspartate in the βC-βD sheet and an arginine[66,91]. These high-resolution protein structures have provided important snapshots of inward rectifiers in different conformational states as well as provided detailed maps for the location of functional domains. The next wave of high-resolution structural analysis should provide a more precise 3-D model of the conformational changes that underlie the gating mechanisms of inwardly rectifying channels.

A macromolecular signaling complex for GIRK channels

Over the last few years, several studies have provided generated for the existence of a macromolecular signaling complex on the plasma membrane. This complex is postulated to contain G proteins, GPCRs, GIRK channels and regulatory proteins92-97 (see review by ref2) (Figure 2C). First, because GIRK channels remain open as long as Gβγ subunits are available, Gα subunits are expected to remain near the channel to terminate GIRK channel activation, through formation of the inactive Gαβγ heterotrimer (Box 1). Second, PTX-sensitive Gα subunits can associate directly with GIRK channels and alter channel gating[84,85,98,99]. In fact, Gα may also be involved in establishing receptor specificity such that stimulation of GPCRs that couple to only PTX-sensitive G proteins activate GIRK channels[84,100]. Third, spectroscopic studies with fluorescently tagged GPCRs, G proteins and regulator of G protein signaling (RGS) proteins, have provided details on the spatial relationship between proteins within this macromolecular signaling complex[26,92,94,96,101,102] (see Box 2). Interestingly, spectroscopic studies with Gαiβγ heterotrimers have suggested that G protein activation involves a conformational rearrangement of the Gαi and Gβγ subunits, in contrast to the canonical model whereby Gα and Gβγ subunits physically separate[103]. The ability to undergo rearrangement, however, may be unique to Gαi, since other studies suggest Gαo may dissociate[104-107]. Together, these studies support a view that GIRK channels reside in a macromolecular signaling complex, governed by protein-protein interactions, limited diffusion and lipid domains[2].

Recent studies have begun to investigate the regulation and localization of GIRK channels expressed in their native environment. In cerebellar granule cells, biochemically restricting the movement of μ opioid receptors does not alter the rate of GIRK activation, revealing that these signaling proteins are localized in membrane compartments and are not freely diffusing in the membrane[97]. Using neuronal PC12 cells, it was discovered that chronic stimulation of endogenous M_2 muscarinic (M_2R) receptors leads to down regulation of both the receptors and GIRK channels[108], indicating M_2R and GIRK are co-regulated in a complex. Furthermore, the $GABA_BR2$ subunit has been shown to dimerize with the M_2R and form an even larger functional $M_2R/GABA_BR2$/GIRK heteromeric complex[109].

Electron microscopic (EM) immunohistochemical studies with brain tissue provide information on the localization of native GIRK channels. In hippocampus, GIRK channels are expressed on the dendritic shafts of hippocampal neurons[110], which has been confirmed by cell-attached patch-clamp recordings111. In addition to the shaft, immuno-gold labeling show a tight association of GIRK channels and $GABA_B$ receptors on dendritic spines[110]. In the cerebellum, immunoelectron microscopy studies show that GIRK2/GIRK3 channels are localized in the postsynaptic density, while GIRK1/GIRK3 channels are perisynaptic[112]. It is tempting to speculate that the subcellular localization may correlate with the Gβγ affinity (GIRK2/GIRK3; lower EC_{50}) in neurons.

Future immunoelectron or super-resolution microscopy studies may reveal additional details on the localization of GIRK channels, $GABA_B$ receptors, RGS and G proteins in neurons, that provide further insights into their function. Discussions concerning the existence of a GIRK macromolecular signaling complex *in vivo* still continue, however. Additional studies will need to clarify the role of collision coupling and whether changes in GIRK signaling occur at the level of the GPCR/GIRK macromolecular complex or individual components in the signaling pathway.

Reducing excitability: physiological roles for GIRK channels

The physiological activation of GIRK channels can shape the neuronal network behavior in many areas of the brain at different levels. The basal activity of GIRK channels contributes to the resting potential of neurons, shifting the membrane voltage by ~−8 mV[27]. This hyperpolarization of the resting membrane potential decreases electrical excitability. In addition, receptor activation of GIRK channels provides another level of inhibition, to which three different changes in signaling can be generally ascribed.

Neuronal self-inhibition

Some neurons release a neurotransmitter to activate Gi/o-coupled receptors and in turn GIRK channels on their own dendrites (autaptic transmission), causing self-inhibition (Figure 3A). For example, a train of action potentials fired by low threshold spiking (LTS) interneurons of the cortex results in long-lasting hyperpolarization. This change in excitability occurs in a cell-autonomous way through endocannabinoids released from dendrites and the activation of CB1 receptors coupled to GIRK channels on the very same dendrites[113]. This modulation of LTS neurons may lead to long-lasting changes in cortical networks owing to altered glutamate transmission in pyramidal neurons. Dopamine neurons also exhibit a form of autaptic inhibition. Here, dendodendritic release of dopamine activates dopamine D2 receptors coupled to GIRK channels, leading to suppression of firing114.

Neuron-to-neuron inhibition

Activation of postsynaptic $GABA_B$ receptors115, D2 receptors116 and group II metabotropic glutamate receptors mGluRs[117] by transmitters released from neighboring neurons (synaptic transmission) may also activate GIRK channels. For GABAergic synapses, GABA released spontaneously or driven by a single action potential produces a fast inhibitory postsynaptic potential, mediated by $GABA_A$ channels (Figure 1C - left panel, 3B). By contrast, strong or repetitive stimulation is required to elicit the slow inhibitory potential, suggesting that GABA released into the synaptic cleft diffuses and activates peri-synaptic $GABA_B$ receptors coupled to GIRK channels (Figure 1C-right panel, 3B)[118-120]. Thus, $GABA_B$ receptors/GIRK channels could be positioned directly adjacent to synaptically localized $GABA_A$ receptors or in a neighboring dendritic spine (for review, see ref121). The slow time course of outward current through GIRK channels therefore correlates with the time required for diffusion of the neurotransmitter and is not limited by

the speed of G protein activation. The reuptake/degradation of the neurotransmitter and the limited space for diffusion may affect the amplitude of the slow IPSC, thus regulating the degree of inhibition. In the hippocampus, generation of the slow IPSC is important for regulating the rhythmic activity of the network119. In midbrain DA neurons, slow activation of GIRKs via D2 receptors originates from local pooling of dendrodendritically released DA114,122. Future studies are needed to demonstrate a physiological role for the slow IPSC *in vivo*. In addition to postsynaptic activation, recent characterizations of $GABA_B$ receptor mediated presynaptic inhibition has revealed a component that is sensitive to a GIRK-specific channel inhibitor tertiapin[123], suggesting a presynaptic role for GIRK channels in inhibiting neurotransmitter release[2,124,125]. Thus, GABA may inhibit pre-synaptic release concurrent with postsynaptic hyperpolarization.

Network-level inhibition

The regulation of ambient levels of several endogenous GPCR agonists (e.g. adenosine, somatostatin) may exert a modulatory effect on large-scale neuronal networks through GIRK channel activation. This large-scale effect of neuromodulators is known as volume transmission. Moderate activation of GIRK channels in a population of neurons would be expected to dampen membrane excitability (Figure 3C). For instance, somatostatin acting via SST5 receptors may alter the oscillation behavior of thalamic networks through post-synaptic activation of GIRK channels, along with presynaptic inhibition[126]. Similarly endogenous adenosine may suppress gamma oscillations in the hippocampus[127], possibly due to the selective expression of A1 receptors on pyramidal neurons, which also activate GIRK channels. Dopamine concentrations are difficult to measure after synaptic or dendritic release[128] but can be modeled in the extracellular space based on known diffusion properties and uptake efficiency of surrounding cells. The transmitter encounters predominantly extrasynaptic DA autoreceptors on DA axons and heteroreceptors on neighboring cells. In this model the sphere of influence for high affinity DA receptors has a volume of several tens of μm^3, and may therefore affect thousands of synapses of many neurons simultaneously. Conditional transgenic mouse lines or cell specific knockouts of GIRK channel subunits are among the future studies that are required to specifically investigate the role of GIRK channels in signaling pathways beyond the direct hyperpolarization.

Moving channels to modulate GIRK

Recently, a plasticity of the slow IPSCs in response to $GABA_B$ and D2 receptor activation has been observed (Figures 3b, 4). For example, depolarization of the postsynaptic neurons to bring the membrane potential close to 0 mV, increases the $GABA_B$ receptor-mediated IPSC several fold for the duration of the experiment[129]. This increased IPSC is independent of the activation of $GABA_B$ receptors, but dependent on NMDAR activation, similar to hippocampal NMDAR-dependent LTP of AMPAR-mediated EPSCs. It has been suggested that GIRK currents become larger because additional channels are inserted130. Indeed, the activation of NMDA receptor in cultured dissociated hippocampal neurons increases the level of the surface expression of GIRK1 and GIRK2 channel in the soma and dendrites within minutes[130]. This insertion requires protein phosphatase-1-mediated dephosphorylation of a GIRK2 serine residue (Ser-9) that promotes channel recycling. Increased expression of GIRK channels would reduce excitability, dampening signaling of hippocampal pyramidal neurons. For example, GIRK channel activation is implicated in the maintenance of LTP at glutamatergic synapses. In mice lacking GIRK2, the depotentiation of LTP in hippocampal neurons is impaired because adenosine A1 receptor fail to activate of GIRK channels [131].

In contrast, activation of dopamine D2 receptors causes a long-term depression (LTD) of the DA-dependent slow IPSC [116]. This LTD is inhibited by the presence of the calcium chelator

BAPTA in the recordings pipette, and seems therefore to rely on a cell-autonomous postsynaptic effect (Figure 3A). This reduction in inhibition would be expected to increase burst firing and DA release in the terminal projections *in vivo*. Less is known concerning the mechanism of this plasticity but could involve desensitization of D2 receptors, modulation of G-protein function and/or GIRK channel availability (as GIRK channels can rapidly redistribute).

Taken together, GIRK channels play a key role in many types of neuronal communication and, as will be outlined in the following sections, modification of GIRK channels can modulate CNS function in health and disease (Figure 4).

GIRK channels in pain perception and analgesia

GIRK channels have been implicated in pain perception. This notion is based on studies with mice carrying mutations in GIRK channels, and the activation of GIRK channels by endogenous pain modulators such as endorphins, endocannabinoids and analgesic drugs.

GIRK channels were first implicated in pain perception when opioid analgesia was tested in *weaver* mice that carry a spontaneous mutation in the pore region of GIRK2[132] leading to a loss of K^+ selectivity and G-protein insensitivity ($GIRK2^{wv}$)[22,133,134]. Upon receptor activation $GIRK2^{wv}$ depolarizes rather than hyperpolarizes neurons. Morphine and a selective κ opioid agonist (U-50488) that are known to activate GIRK2-containing channels through opioid receptors reduce pain perception in wild-type mice, but not in the *weaver* mice[135]. The interpretation of the opioid-dependent behaviors, however, is confounded by the loss of neurons in the substantia nigra and cerebellum[136].

Subsequent studies have taken advantage of GIRK channel knockout mice (see Table 1). In $GIRK2^{-/-}$ mice, for example, the dose-response curve for morphine and clonidine (an α2AR agonist) analgesia is shifted to higher concentrations, while the maximal effect was preserved137. This finding was confirmed in a recent study using a GIRK2/3 double knockout mouse30; in these mice the potency (coupling efficiency) of opioid-analgesia was reduced, but their efficacy (maximal response) was intact.

Knockout studies also suggest that GIRK channels may account for gender difference in nociception. The higher pain threshold in males, measured as a longer latency for tail withdrawal to radiant heat, is abolished in $GIRK2^{-/-}$ mice[137], indicating that GIRK channel activation may account for some of the gender differences in normal pain perception. Further evidence for this notion came from a study that showed the analgesic effects of ethanol, oxotremorine (muscarinic receptor agonist), baclofen ($GABA_B$ receptor agonist), clonidine (α2 receptor agonist) and WIN 55,212-2 (cannabinoid receptor agonist) reduced or eliminated in male but not female GIRK2−/− mice[138]. Studies using mice lacking GIRK1 or GIRK2 channels show a blunted response to μ- ([D-Ala(2),N-Me-Phe(4),Gly(5)-ol]-enkephalin, DAMGO) and ∂-(Tyr-D-Ala-Phe-Glu-Val-Val-Gly amide) but not κ-opioid receptor ((trans)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methanesulfonate hydrate) agonists when applied intrathecally139. Similarly, intrathecal tertiapin, a selective GIRK channel inhibitor123, reduced the efficiency of DAMGO to increase the latency for withdrawal in the immersion tail-flick test. Therefore, while further studies are needed to clarify the role of GIRK channels in opioid-induced analgesia, there is general agreement that GIRK channels modulate systemic opioid (or G_{io})-mediated analgesia through postsynaptic inhibition.

The periaqueductal grey (PAG), a small nucleus in the brain stem, has a crucial role in central analgesia; direct application of opioids into the PAG inhibits GABA neurons through both GIRK-independent (presynaptic) and GIRK-dependent (postsynaptic) mechanisms,

which is sufficient to induce analgesia[140,141]. The disinhibition of principal neurons of PAG that project to nuclei of the caudal brain stem and modulate ascending pain pathways likely plays a significant role in the analgesic effects of opioids.

GIRK channels have also been implicated in opioid tolerance and dependence due to adaptive changes in chronic exposure to morphine. However, opioid receptor agonist-induced desensitization of GIRK currents seems to involve additional mechanisms. In locus coeruleus neurons, inhibiting both G protein receptor kinase 2 (GRK2) and extracellular signal-regulated kinase (ERK) signaling significantly reduces the met-enkephalin induced GIRK channel desensitization that normally occurs within minutes of drug application[142], an adrenergic nucleus in the pons that lowers pain threshold when activated. However, GIRK channel desensitization may not occur when μ-opioid receptors are activated by morphine. In fact, morphine elicits much less GIRK channel desensitization *in vitro* and *in vivo*[143] compared to met-enkephalin. This response is surprising because met-enkephalin induces much less drug tolerance and dependence, than morphine. A recent report even suggests that the potency of morphine to reduce pain (i.e. the effect at a submaximal concentration) may actually increase during the development of tolerance (i.e. reduction of maximal effect) [144]. This is paradoxical but could be explained if tolerance and dependence are mediated by effectors of opioid receptors other than GIRK channels.

An alternate role of GIRK channels in opioid dependence is suggested by the following observations. Mice lacking GIRK2 and GIRK3 have strongly reduced withdrawal signs normally induced in wild-type controls by the opioid receptor antagonist naloxone after chronic exposure to morphine. In the mutant mice, the withdrawal syndrome can be rescued if the output from locus coeruleus neurons is inhibited during the exposure to morphine[30]. This suggests that GIRK channels 'gate' the induction of opioid dependence by silencing the neurons in the locus coeruleus every time opioids are injected.

Taken together these data suggest that GIRKs have a role in pain perception under specific conditions, and that they contribute to opioid-analgesia. However, the literature remains controversial on the role of the PAG and the LC as two key regions in the regulation of opioid-analgesia. Further insight may come from mouse lines in which GIRK subunits are mutated in a cell type-specific manner (e.g., only GIRK-expressing neurons in the locus coeruleus) or where GIRK channels are coupled to light-activated GPCRs.

Altered excitability and dying neurons: GIRK channels in disease

GIRK channels are implicated in the pathophysiology of several diseases, including epilepsy, addiction, Down's syndrome, ataxia and Parkinson's disease. Two broad principles have been distinguished. First, loss of GIRK function can lead to excessive excitability leading, such as in epilepsy, while a gain of GIRK function can significantly reduce neural activity, as postulated in Down's syndrome. Second, loss of selectivity can cause ion fluxes across GIRK channels, such as excessive K^+ efflux that triggers cell death, exemplified in a model of Parkinson's disease.

Changes in excitability

The involvement of GIRK channels in epilepsy has been inferred from the phenotype of mice where a GIRK subunit has been deleted. GIRK2 knockout mice develop spontaneous convulsions and show a propensity for generalized seizures when injected with a pro-convulsive $GABA_A$ receptor antagonist[145]. Drugs such as desimipramine, fluoxetine, haloperidol, thioridazine, pimozide and clozapine[146] (Supplemental Table S1) that are used for the treatment of various neurological disorders, may inhibit GIRK channels and hence cause seizures, a known side effect of these drugs. On the other hand, electroconvulsive

shock leads to increased expression of GIRK channels[147], which may serve as a possible neuroprotection for inappropriate activity. In support of this idea, stimulation of galanin type 2 receptors that activate GIRK channels prevents kindled epileptogenesis in rats[148], highlighting GIRK channels as a novel target for anticonvulsants.

Down's syndrome is a genetic disorder caused by duplication of human chromosome 21, which contains the *Girk2* (*Kcnj6)* gene. To investigate the contribution of a third copy of the *Girk2* gene to the phenotype of Down's syndrome, two mouse models have been generated that carry either a partial or full segment duplication of the mouse chromosome 16, the ortholog to human chromosome 21[149,150]. In both lines, GIRK2 protein is upregulated, resulting in a larger slow IPSC mediated by $GABA_B$ receptors[151]. Moreover, hippocampal LTP is reduced while LTD is enhanced in both lines and therefore synaptic plasticity of glutamatergic transmission is altered. Since the duplicated chromosomal segment also carries other genes, additional studies are needed to specifically test the role of GIRK channels in the malfunction of the nervous system in Down's syndrome.

Addiction

GIRK channels are thought to have a role in the acute rewarding effects and/or the adaptation that occurs with chronic exposure to addictive drugs that work through GPCRs, such as opioids, the club-drug GHB, and cannabinoids. Addictive drugs are known to strongly increase dopamine levels in the mesocorticolimbic system. Based on *in vivo* and *in vitro* experiments, distinct cellular mechanisms have been proposed for different classes of drugs[152]. Opioids and the club drug γ-hydroxbutyrate (GHB) activate GIRK channels leading to disinhibition of DA neurons. Morphine stimulates μ-opioid receptors that are selectively expressed on interneurons of the VTA and activate GIRK channels, reducing the firing of these cells, eventually leading to disinhibition of DA neurons[153]. The effect of GHB is more complex, as $GABA_B$ receptors are expressed on both GABA and DA neurons. However, DA neurons in the VTA are an order magnitude less sensitive (higher EC_{50}) to $GABA_B$ receptor agonists than GABA interneurons[43] most likely due to the selective expression of GIRK2c and GIRK3 and the lack of GIRK1 subunit (see discussion above). Consequently, low concentrations of GHB only activate GIRK currents in GABA neurons (containing GIRK1 subunits), leading to disinhibition of DA neurons. In addition, DA neurons in the VTA also selectively express RGS2, which contributes to the lower $GABA_B$R-GIRK sensitivity[26]. Interestingly, chronic exposure to morphine or GHB enhances the coupling efficiency (EC_{50}) for $GABA_B$ receptors and GIRK channels by lowering levels of RGS2 in DA neurons[26]. Under these circumstances the concentration window for disinhibition is much narrower and now low concentrations of drug are sufficient to inhibit DA neurons and become behaviorally aversive26. Thus, the combination of GIRK subunits expressed in a neuron can determine the signaling properties of neuronal circuits. In the case of cannabinoids, the disinhibition of DA neurons seems to occur primarily through presynaptic, GIRK-independent mechanisms154 (but see 124,125).

GIRK channels may also be involved in the response to psychostimulants, such as cocaine and amphetamines. SNX27, a psychostimulant-inducible protein[155], specifically regulates surface expression of GIRK2c and GIRK3[40]. GIRK2 and GIRK3 knockout mice exhibit dramatically reduced intravenous self-administration of cocaine relative to wild-type mice[35], while surprisingly, the GIRK2/3 double knockout self-administer more cocaine than wild type mice suggesting compensation in the single subunit knockouts that is no longer possible in the double knockout. Elucidating the changes in GIRK signaling with psychostimulants is an exciting area for future research and may lead to further insight of the role of GIRK channels in addiction.

Because ethanol directly binds and activates GIRK channels[46-48,156], they may mediate some ethanol-related behaviors. In fact, ethanol has been shown to enhance $GABA_B$-activated GIRK currents in neurons of the VTA[156], which would decrease the firing frequency of DA VTA neurons in rodents. As discussed above, GIRK channels likely have a role in the analgesic effects of ethanol138›157, but whether they also contribute to the reinforcing effects of ethanol is less clear. GIRK2 knockout mice exhibit reduced conditioned taste aversion, compared to wild-type mice suggesting that GIRK activation could be involved in the motivational response of ethanol158,. However, these differences were observed with moderate but not high doses of ethanoll[158]. Using a different approach, involving identification of a quantitative trait locus (QTL) with large effects on predisposition to physical dependence and associated withdrawal from sedative hypnotics such as ethanol, a 0.44 Mb region of mouse chromosome 1 was mapped for this QTL that contains *Girk3* (Kcnj9)[34]. In a behavioral assay designed to measure withdrawal, GIRK3 −/− mice show less severe withdrawal symptoms from ethanol and barbituarates use34.

Based on these results, new approaches for addiction therapies have emerged and more may come. For example, pharmacological activation of GIRK channels by administration of the $GABA_B$ receptor agonist baclofen reverses behavioral sensitization to morphine in rats[159] and clinical *Phase 2* & 3 trials are underway examining the effect of baclofen for treating ethanol and cocaine addiction.

Cell death due to GIRK signaling

Parkinson's disease (PD) is a debilitating motor coordination disorder caused by degeneration of dopamine neurons in the substantia nigra (SN). The involvement of GIRK channels in Parkinson's disease was first inferred from the *weaver* mouse[132], where constitutively active $GIRK2^{wv}$ channels produce chronic depolarization and cell death in a subset of neurons in the brain[22,133,134] mimicking the neuronal degeneration observed in Parkinson's disease[160]. The gain-of-function phenotype in DA neurons of *weaver* mice is of clinical interest due to the progressive degeneration of DA neurons in the substantia nigra, while DA neurons in the VTA are spared in the early stages of the disease[161]. Interestingly, DA neurons of the SN express only GIRK2, which results in a gain of function, while DA neurons in the VTA coexpress GIRK2/GIRK3, which may produce a loss of function due to co-assembly with GIRK3[22,133]. Other mechanisms of DA cell death in the *weaver* mouse have been proposed, however. One study has suggested that constitutively active $GIRK2^{wv}$ channels trigger activation of K-ATP (Kir6.2) channels in response to mild mitochondrial uncoupling in SNc DA neurons but not VTA DA neurons[162] while other studies suggest a general susceptibility to oxidative stress (due to reduced thiol redox state) in DA neurons163. *Weaver* mice have additional developmental abnormalities that result in a complex phenotype including learning deficits, epileptic seizures, and motor disturbances (e.g. ataxia).

Another line of evidence implicating GIRK channels comes from a recent study investigating nerve growth factor (NGF) mediated programmed cell death in dorsal root ganglion neurons, an important step in the proper normal development of the nervous system. This study shows that activation of the neurotrophin receptor p75 NTR increases plasma membrane levels of PIP_2, activating GIRK2 channels and creating a sustained K^+ efflux that stimulates programmed cell death[164].

Taken together, GIRK channels may contribute to the pathophysiology of a variety of human diseases, either by altering the excitability of specific neurons, changing the slow IPSCs, or promoting cell death. It is important to note that the suggested roles for GIRK channels in brain diseases are based on observations in animal models. Future genetic studies may provide direct evidence for altered GIRK function in human diseases.

Conclusions

Research over the last twenty years has elucidated the molecular and structural determinants of GIRK channel activation and modulation by G proteins (Gα andGβγ, with the emerging concept of the existence of macromolecular signaling complexes. Knockout mice for all four mammalian subunits greatly contributed to the understanding of GIRK functions, namely to regulate the excitability of neurons in a cell autonomous fashion, in-between neighboring neurons by regulating synaptic transmission and also regulating the activity of large-scale networks. While we only begin to understand the role of GIRK channels with different subunit compositions, their respective expression pattern in the brain will give further functional insight. For example, DA neurons of the VTA express a unique GIRK2/3 heteromeric channel that has a particularly low affinity for the Gβγ dimer. More research is needed to link such cellular observations to function and behavior. The development of novel tools, such as light activation of GPCRs (optoXRs)[165] that couple to GIRK channels as well as conditional and cell-specific knock-out mouse lines, will be important to further advance our understanding of GIRK function. These tools will also be crucial for the investigation of GIRK channels in disease and may help to design specific drugs, selectively opening or closing GIRK channels composed of different subunit compositions, to treat addiction, epilepsy, Down's syndrome, or Parkinson's disease.

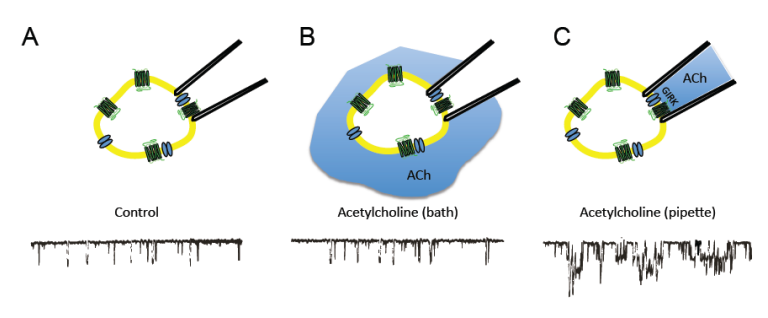
Box 1

The great debate of the 1980s: activation of GIRK channels by Gβγ or Gα G proteins?

Over 20 years ago, an impassioned debate arose over the mechanism of GIRK channel activation. At that time, the canonical pathway for G protein signaling involved exchange of GTP for GDP on the Gα subunit, and subsequent Gα-GTP dependent activation or inhibition of catalytic enzymes, such as adenylyl cyclase or phospholipase C. Cardiac GIRK channels, referred to as I_{KACh}, on the other hand were hypothesized to be activated directly by G proteins through a membrane delimited pathway[4,166]. One of the first hints of a novel signaling pathway came from studies using patch-clamp recordings from rabbit atrial cells: bath application of acetylcholine (ACh) did not alter GIRK channel activity recorded in cell-attached patches (panel b), but when ACh was included in the recording pipet (panel c), GIRK channel activity greatly increased (taken with permission from ref166). These results suggested that activation of GIRK channels by G protein coupled receptors occurs through proteins anchored to the membrane, and not through freely diffusible cytoplasmic 2nd messengers.

Two different groups examined whether GIRK channels were activated directly by G proteins. One group showed that application of purified native or recombinant Gα pre-activated with GTPγS (Gα-GTPγS) led to robust GIRK channel activation[167,168]. Another group found that application of purified Gβγ subunits, and not Gα-GTPγS, directly activated GIRK channels[5]. Thus, there was agreement on the direct activation of GIRK channels by G proteins but little consensus on whether Gα or Gβγ subunits mediated receptor activation. With the cloning of GIRK subunits and improved protein expression and purification techniques, two papers in 1994 provided new evidence for Gβγ-dependent activation. In one study, expression of recombinant Gβγ subunits led to sustained activation of GIRK channels, which was attenuated by a novel type of Gβγ binding protein, βARK7. A second study showed that different combinations of recombinant and purified Gβγ dimers directly activated GIRK channels[6]. Today, there is agreement that Gβγ subunits activate GIRK channels and, although there have been no further experiments demonstrating that Gα activates GIRK channels, Gαi/o G proteins

are still considered important regulators of GIRK channels affecting receptor specificity and basal channel activity[82,84,85,92,99,100,169].



Box 2

Spectroscopic studies provide evidence for dynamic GIRK/GPCR macromolecular complex

High-resolution spectroscopic studies measuring fluorescence resonance energy transfer (FRET) have been used to investigate the spatial proximity of proteins within the macromolecular complex. FRET occurs when two fluorophores with overlapping spectral properties are aligned and <100 Å away from each other. If there is movement in the protein such that the distance (or orientation) between the two fluorophores changes, then FRET will increase when they move closer or decrease when they move apart. In a macromolecular complex, one would predict that if the proteins are physically close then FRET measurements can be used to determine whether two specific proteins are closer than 100 Å of each other. FRET studies have indicated that some GPCRs pre-couple with G proteins and channels94،96،101 (but see170). $GABA_B$ receptors and RGS proteins also produce detectable FRET with GIRK channels26،102. Using a similar technique, a significant bioluminescence resonance energy transfer (BRET) signal has been detected between tagged β2 adrenergic receptors and GIRK channels[96]. Recently, a FRET study using TIRF microscopy (Total Internal Reflection Fluorescence) and tagged GIRK channels and Gβγ subunits surprisingly found that FRET efficiency decreased upon receptor activation92 – one would have expected FRET to increase as Gβγ and GIRK channels come closer together. These findings support the model that the Gαβγ heterotrimer associates directly with GIRK channel at rest and that Gβγ moves within a restricted region around the channel during activation. Collectively, these studies have provided important evidence for the existence of a macromolecular signaling complex containing GIRK channels, GPCRs, G proteins and RGS proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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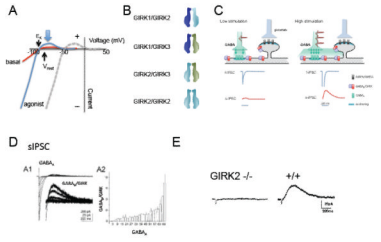

Figure 1. Physiology of GIRK channels in the mammalian brain

A, Hypothetical example of inwardly rectifying potassium currents through GIRK channels. Current (I) is plotted as a function of voltage (V) (I-V plot). With physiological levels of extracellular K, the current reverses (zero current potential) near −90 mV at the equilibrium potential for K (E_K). The basal current (red) and agonist-induced (blue) currents show inward rectification (large inward (−) and small outward current (+)). The outward flow of current near the resting potential of the cell (large arrow) hyperpolarizes the neuron's membrane potential. With higher extracellular potassium (e.g. 20 mM KCl), a concentration commonly used to study GIRK channels, the I-V relation shifts to the right to the new E_K (dashed line), demonstrating a universal property of inwardly rectifying K channels. **B**, Three primary GIRK subunits exist in brain and form heterotetramers (GIRK1-GIRK2, GIRK2-GIRK3, GIRK1-GIRK3) and homotetramers (GIRK2-GIRK2)[3,19-23]. Less is known about GIRK4 in the brain. **C**, Generation of the slow IPSC. Two levels of synaptic activity are shown. Low stimulation of a GABA-ergic neuron releases GABA at levels sufficient to only activate $GABA_A$ channels (left panel). Higher stimulation (right panel) releases more GABA that accumulates in the synaptic cleft and diffuses to neighboring $GABA_B$/GIRK complexes, located on the shaft and dendritic spine[118-120]. The resulting hypothetical fast and slow IPSCs from these two scenarios are shown below. **D**, Electrophysiological recording of the fast IPSC ($GABA_A$) and slow IPSC ($GABA_B$-GIRK) from a hippocampal slice (taken with permission from ref119). The relationship between the $GABA_A$ and $GABA_B$ response is non-linear (A2), suggesting accumulation of GABA is needed to activate $GABA_B$ receptors. **E**, The $GABA_B$ receptor dependent sIPSC is absent in the hippocampus of mice lacking GIRK2 channels (taken with permission from ref27).

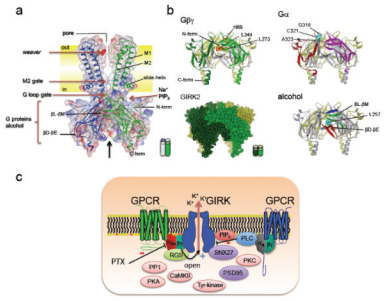

Figure 2. Structural insights into gating and the formation of a macromolecular GIRK signaling complex

A, A structure (ribbon model superimposed with space filled model) of the Kirbac1.3/GIRK1 chimeric channel[72] is shown in a typical lipid bilayer ('out': extracellular) This structure contains the cytoplasmic domains (N- and C-termini) of GIRK1, and the transmembrane domains (M1, M2) and pore region of the bacterial inward rectifier (Kirbac1.3) channel (PDB:2QKS). The structure of the GIRK1 cytoplasmic domain in this full-length chimeric protein is similar to those of other inward rectifiers 64-66. The K^+ selectivity filter is the site of the *weaver* mutation[22,133,134]. The regions implicated in Na^+ and PIP_2 association are shown[58-60,66,91]. Two channel gates formed by the M2 transmembrane domain and cytoplasmic G-loop form a physical barrier to ion permeation[65,67,68,88-90]. Note the constriction formed by the G-loop gate[65]. **B**, Modulation sites are shown from a side-view perspective in two adjacent subunits. The regions implicated in Gβγ activation (light and dark green β-strands; H69, L273 and L344: Yellow)9,75-82, Gαi/o association (red and pink β-strands, G318, C321 and A323 - blue)80,84,85, and ethanol-dependent activation (N-terminus:blue; βD-βE sheet: yellow; βL-βM sheet: green; L257:red)46 are shown. The sites are mapped on structure of GIRK2 (PDB:2E4F)66 **C**), Schematic shows a macromolecular signaling complex that contains a GPCR, which couples to PTX-sensitive Gαi/o G proteins, a GIRK channel, an RGS protein, SNX27, and possibly PSD95. Gβγ opens GIRK channels (+). Modulators of GIRK channels are also shown, including tyrosine kinase, CaMKII kinase, PKA/PKC kinases, and PP1 phosphatase[49-57,129,130,171]. A second GPCR that couples to the Gq pathway is shown. Activation of this pathway stimulates PLC, which leads to activation of PKC, and depletion of PIP_2, both of which reduce (−) GIRK channel activity55,61,62. Pertussis toxin (PTX) inhibits (−) activation of GIRK channels through Gαi/o G proteins4.

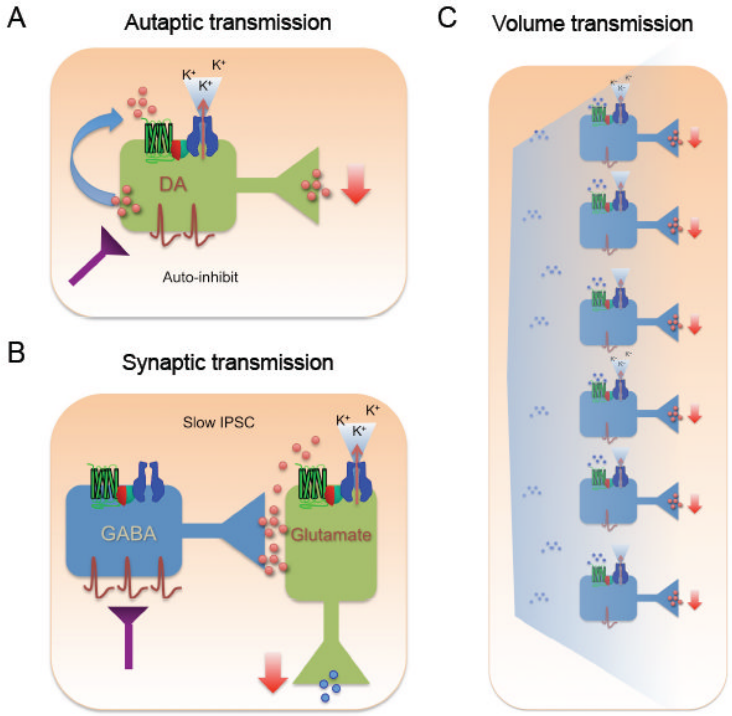

Figure 3. Three types of neuronal signaling pathways for GIRK channels in the brain

A) In autaptic synapses, neurotransmitters are released by and bind to the same neuron, stimulating GPCRs and activating GIRK channels. The net effect of such an autapic synapse is a reduction in the release of neurotransmitter (negative feedback). **B**) The slow IPSC. High stimulation triggers release of GABA that spills over to neighboring $GABA_B$ /GIRK complexes, located on the dendritic spine and shaft. **C**) Network modulation. GIRK channels involved in volume transmission. When a neurotransmitter is released from many neurons, the ambient concentration rises and diffuses to activate GIRK channels on target neurons. This leads to reduced network activity.

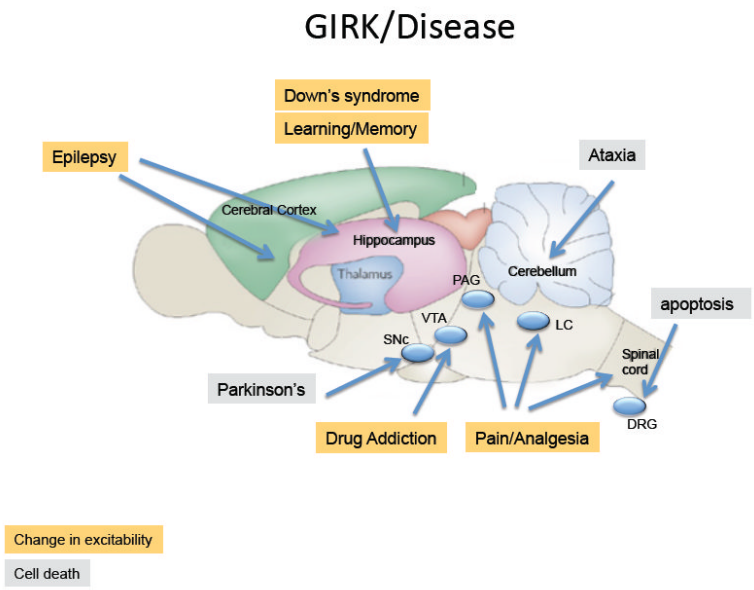

Figure 4. GIRK channels are implicated in different disease states

Schematic shows saggital view of a rat brain highlighting regions where GIRK channels have been implicated in brain neuronal disorders or diseases due to changes in excitability (yellow) or cell death (grey). Changes in GIRK activity throughout the brain may contribute to: *Epilepsy*: GIRK2 knockout mice develop spontaneous convulsions and show a propensity for generalized seizures[145]. Similar pro-convulsive effects are observed with GIRK channel inhibitor tertiapin when administered intrathecally.**]**[148]. *Down's Syndrome & Learning and Memory*: Two mouse models for Down's syndrome, the Ts65Dn[149] and the Ts1Cje DS[150], contain a gene duplication for *Girk2* and show larger slow IPSC mediated by $GABA_B$ receptors[151], impairments in LTP and enhancement of LTD151. Although all neurons are affected, this phenotype may involve mostly hippocampal functions. *Drug Addiction:* GIRK knockouts show impairment in self-administration of cocaine[35], altered response to GHB[26], less severe withdrawal from sedatives[34] and reduced conditioned place taste aversion for ethanol[158]. Changes in the function of the mesolimbic system, involving the VTA, are thought to underlie these changes. *Pain:* Removing GIRK2/GIRK3 reduces the potency (coupling efficiency), but leaves efficacy (maximal response) of opioid-analgesia intact[30,137], effects most likely involving the peri-acqueductal grey and spinal cord. Chronic neuropathic pain can lead to tyrosine phosphorylation of GIRK1 subunits, which reduces basal GIRK activity171. *Ataxia/Parkinson's*: In the *weaver* mouse, GIRK2 channels contain a mutation that eliminates K+ selectivity and leads to degeneration of midbrain SNc DA neurons and cerebellar granular neurons[161]. The gain-of-function phenotype in DA neurons is of clinical interest due to its similarity to the degeneration in Parkinson's disease. In dorsal root ganglion (DRG) cells, activation of the neurotrophin receptor p75 NTR increases levels of PIP_2, which activates GIRK2 channels and signals to promote programmed cell death via K^+ efflux-induced apoptosis[164].

Table 1

Summary of GIRK knockout mice

GIRK knockout	Phenotype								Generator
	Agonist-evoked GIRK current							Behavioral	
	Brain						Heart		
	VTA	SNc	CA1	LC	Spinal cord L II	Cerebellum	Atrium		
GIRK1$^{-/-}$	nc [26]	nc [28]	↓ [28]		↓ [31]		↓ [172]	• Reduced score in anxiety behavior testing [33]	172
GIRK2$^{-/-}$	↓ [26]	↓ [28]	↓ [27,28]	↓ [29,30]	↓ [31]	↓ [32]		• Reduced anxiety with signs of hyperactivity [33,173] • Gender difference in latency for tail withdrawal to radiant heat abolished [137,138] • Reduced cocaine self administration [35] • Reduced conditioned taste aversion [158] • Spontaneous convulsions and propensity for generalized seizures [145,174]	145
GIRK3$^{-/-}$	↓ [26]	nc [28]	nc [28]	nc [29] ↓ [30]	nc [31]			• Decreased morphine and clonidine analgesia [137] • Less severe withdrawal from sedatives [34] • Reduced cocaine self administration [35]	29
GIRK2$^{-/-}$ GIRK3$^{-/-}$	↓ [26]		↓ [28]	↓ [29,30]				• Attenuation of morphine withdrawal [30] • Propensity for spontaneous and lethal seizures between 2 and 8 months of age [29] • Increased cocaine self-administration [35]	29
GIRK4$^{-/-}$							↓ [175]	• Impaired performance in the Morris water maze [10]	175

Table 2

Brain G protein-gated Inward Rectifiers

Brain Region	Single-channel conductance	Composition
hippocampal pyramidal[111,176,177]	33-38 pS	GIRK1, 2a, 2c, 3
substantia nigra[41,178]	56 pS	GIRK2a, 2c
locus coeruleus[179,180,182]	30 & 45 pS	GIRK1, 2, 3, 4
nucleus basalis[181,182]	32-39 pS	GIRK1,2,3 4
ventral tegmental area[43]	unknown	GIRK1, 2c, 3
medial prefrontal cortex[57]	30 pS	unknown