Tinnitus: Prospects for Pharmacological Interventions With a Seesaw Model

Hannah Tetteh¹, Minseok Lee², C. Geoffrey Lau¹, Sunggu Yang², and Sungchil Yang¹

Abstract
Chronic tinnitus, the perception of lifelong constant ringing in ear, is one capital cause of disability in modern society. It is often present with various comorbid factors that severely affect quality of life, including insomnia, deficits in attention, anxiety, and depression. Currently, there are limited therapeutic treatments for alleviation of tinnitus. Tinnitus can involve a shift in neuronal excitation/inhibition (E/I) balance, which is largely modulated by ion channels and receptors. Thus, ongoing research is geared toward pharmaceutical approaches that modulate the function of ion channels and receptors. Here, we propose a seesaw model that delineates how tinnitus-related ion channels and receptors are involved in homeostatic E/I balance of neurons. This review provides a thorough account of our current mechanistic understanding of tinnitus and insight into future direction of drug development.

Keywords
acoustic trauma, homeostatic plasticity, sensory loss, phantom pain, auditory pathway

Introduction
Tinnitus is the perception of sound without the presence of external acoustic stimuli. The sound perceived by sufferers is generally subjective and described as a ringing, whistling, clicking, buzzing, or roaring sound. The prevalence of tinnitus varies from country to country in the range between 4.6% and 30.3% (Gilles and others 2013; Jalessi and others 2013; Khedr and others 2010; McCormack and others 2014; Park and Moon 2014; Quaranta and others 1996; Sindhusake and others 2003). Tinnitus can be tolerated but, for about 2% of the patients, it is considered to be severely disabling (Henry and others 2003). Comorbid effects caused by tinnitus such as stress, insomnia, anxiety, depression, deficits in attention, and even suicide, are devastating in everyday life (Cima and others 2011; Crönlein and others 2007; Erlandsson and Hallberg 2000; Gomaa and others 2014; Mazurek and others 2015; Pajor and others 2013; Simoes and Hébert 2012). It has been suggested that tinnitus is usually associated with brain injuries, ageing, noise trauma, emotional and attentional instability (Langguth and others 2013; Martines and others 2015; Shore and others 2016). After being exposed to similar risk factors, some people do not develop tinnitus while others do develop it. This indicates that, other than the environmental risk factors, there is genetic susceptibility to tinnitus (Pawelczyk and others 2012; Sand and others 2011). It is notable that genetic factors are likely associated with chronic tinnitus but not acute tinnitus. Almost everyone develops tinnitus immediately after noise- or drug-induced tinnitus, but few develop chronic tinnitus (Gilles and others 2013; Salvi and others 2009; Widen and Erlandsson 2004). This suggests that chronic tinnitus might have a genetic basis. In line with this, some recent genome-wide association studies revealed a genetic basis for tinnitus (Gilles and others 2017; Vona and others 2017). Nonetheless, the causal association of genetic factors with locus remains to be elucidated.

Despite the various etiologies of tinnitus, it is highly associated with hearing loss. The cellular and neural mechanisms of tinnitus remain unclear. Hence, investigations of animal models where tinnitus is induced by hearing loss have yielded valuable information on the mechanisms of tinnitus (Eggermont and Roberts 2015; Lobanin and others 2006; Yang and Bao 2013). It has been suggested that the
induction of tinnitus is largely due to deafferentation of auditory nerve caused by damage of cochlear hair cells. Deafferentation generally leads to hyperexcitability or enhanced excitation/inhibition (E/I) ratio in central auditory neurons (Jastreboff 1990; Weisz and others 2006). For example, the deafferentation-induced tinnitus can lead to reduced inhibition (Rüttiger and others 2013), increased neural spontaneity (Roberts and others 2010), abnormal synchronous activity (Eggermont 2007), and altered homeostatic plasticity (Yang and others 2012; Yang and others 2011). The auditory areas such as the dorsal cochlear nucleus (DCN) (Koehler and Shore 2013; Shore and others 2008), auditory brainstem (Brozoski and others 2013), inferior colliculus (Mulders and Robertson 2009), auditory thalamus (Caspar and Llano 2017), and auditory cortex (Engineer and others 2011; Llano and others 2012; Yang and others 2011) are associated with tinnitus (Fig. 1). Besides the auditory pathways, other brain regions affected by tinnitus include the limbic system, prefrontal cortex, parietal cortex, cingulate cortex, cerebellum, and subcallosal region (Bauer and others 2013; Boyen and others 2013; Davies and others 2017; De Ridder and others 2011; Langguth and others 2012; Leaver and others 2011; Middleton and Tzounopoulos 2012; Mühlau and others 2006; Rauschecker and others 2010; Roberts and others 2013).

Many attempts have been made to treat tinnitus. Therapeutic drugs alleviating tinnitus have been widely utilized with other treatments such as physical therapy, psychological therapy, acupuncture, and acoustic therapy (Joos and others 2015; Langguth and others 2009; Michiels and others 2016; Park and others 2000; Salvi and others 2009; Scott and others 1985). There are lines of evidence that drug therapies are associated with the function of ion channels and/or neurotransmitter receptors (Elgoyhen and Langguth 2010; Langguth and others 2009; Salvi and others 2009). To pinpoint suitable druggable targets, there is a need to understand the roles of ion channels and receptors in tinnitus. Successful drug therapies require long-term efficacy of drug administration with a lack of adverse side effects, including addiction and biocompatibility. We believe that this can be achieved in the near future by focusing on the contribution of ion channels and receptors to tinnitus. Here we provide a broad overview and summary (Table 1) of the involvement of ion channels and receptors and propose that, by modulating these targets and related plasticity in the auditory system, we can develop new treatments for this debilitating disorder.

**Ion Channels and Receptors in Tinnitus**

Ion channels and ionotropic neurotransmitter receptors have aqueous pores located at the plasma membrane of cells and enable ions to move in or out of cells, thereby altering cellular excitability (Hille 2001; Moody and Bosma 2005). There are various types of ion channels/receptors, and they localize to different subcellular compartments such as dendrites, axons, and somata. They can largely be identified by how they are activated and what types of ions move through them. Ion channels/receptors can be gated by several factors such as membrane voltage change, ligand, second messenger, phosphorylation, pH change, temperature and mechanical distortion. It is well-known that dysregulation of ion channels/receptors results in neural dysfunction by altering neuronal synaptic inputs, excitability, or release properties (Weilinger and others 2013). The following is a list of ion channels/receptors shown to be involved in tinnitus (highlighted in Fig. 1).

**Glutamate Receptors**

Glutamate receptors are the main class of receptors that mediate excitatory transmission in the nervous system (Plested and Mayer 2009). Two major glutamate receptors—AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propionic acid) and NMDA (N-methyl-D-aspartate) receptors—are frequently associated with tinnitus. These receptors contribute to neuronal excitability, plasticity and modulating homeostatic E/I balance (Asztély and Gustafsson 1996; Lau and Zukin 2007; Malenka and Nicoll 1993; Plested and others 2004; Turrigiano 2011; Yang and others 2016).

Glutamate receptor–mediated excitability has been deeply involved in the pathophysiology of tinnitus animal models. In a noise-induced tinnitus animal model, widespread changes in glutamate levels were found along the auditory pathway but in a region-specific manner: Increment in bilateral DCN, bilateral auditory cortex and ipsilateral auditory thalamus; decrement in contralateral auditory thalamus (Brozoski and others, 2012). The application of ifenprodil, an NMDA receptor antagonist, into the cochlea alleviated animal tinnitus behavior of place-tone conditioning (Guittion and Dudai 2007). The cochlear round-window application of another NMDA receptor antagonist, AM-101, reduced the noise-induced loss of ribbon synapses of inner hair cells of AM-101 responders, therein suggesting the reduction of the risk of tinnitus development (Bing and others 2015). Consistent with these findings, tinnitus was attenuated when an NMDAR antagonist, D(-)-2-amino-5-phosphonopentanoic acid (D-AP5) was employed in the rats’ DCN (Brozoski and others 2013). Also, the use of noncompetitive NMDA receptor antagonists, MK-801, 7-chlorokynurenate (7-CK), and gacyclidine, decreased a salicylate-induced tinnitus behavior in rats (Guittion and others 2003; Ruel and others 2008). These studies implicate the significance of NMDA receptors on tinnitus development.
The involvement of NMDA receptors in tinnitus has been also studied with human subjects. When gacyclidine, a noncompetitive NMDAR antagonist, was administered to cochlea of tinnitus patients, 67% of patients experienced temporary recovery from tinnitus (Wenzel and others 2010). In another patient study, oral administration of neramexane, an NMDA receptor antagonist, significantly alleviated tinnitus annoyance and tinnitus impact of life in a dose-dependent manner (Suckfüll and others 2011). Also, caroverine, an AMPA and NMDA receptor antagonist, was able to reduce the severity of tinnitus (Denk and others, 1997). Acamprosate, which is known to reduce the excitatory effect of NMDA receptors, was used to successfully relieve tinnitus symptoms of about 86.9% of subjects (Azevedo and Figueiredo 2005).
<table>
<thead>
<tr>
<th>Class</th>
<th>Subclass</th>
<th>Subject</th>
<th>Region Tested</th>
<th>Tinnitus Induction</th>
<th>Type of Measure</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMDA receptor</td>
<td>Human (18-65 years)</td>
<td>Pure tone audiometry</td>
<td>Dose-dependent improvement by neramexane</td>
<td>Suckfüll and others (2011)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human (18-55 years)</td>
<td>Cochlea Perilymph fistula Viral infection Physical activity Tooth infection Unknown factors</td>
<td>Audiometry Tomography Questionnaire</td>
<td>Temporary relief by gabaccline (4/6 patients)</td>
<td>Wenzel and others (2010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (22-87 years)</td>
<td>Presbycusis Noise trauma Metabolic Otoxicity Autoimmune Idiopathic Multiple</td>
<td>Pure tone audiometry Speech audiometry Immittance audiometry Questionnaire</td>
<td>No significant improvement by memantine</td>
<td>Figueiredo and others (2008)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>NIHL Prebycusis Metabolic</td>
<td>Visual analog scale</td>
<td>Tinnitus relief by acamprosate (86.9%)</td>
<td>Azevedo and Figueiredo (2005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD rats</td>
<td>Al</td>
<td>Salicylate Quinine</td>
<td>Behavior paradigm (SIPAC)</td>
<td>No significant improvement by memantine</td>
<td>Lobarinas and others (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td>DCN</td>
<td>Noise trauma (16 kHz for 1 hour at 120 dB)</td>
<td>Behavior paradigm (Operant conditioned-suppression)</td>
<td>Reduction of neural activity by DAP5</td>
<td>Brozoski and others (2013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar rats</td>
<td>Cochlea</td>
<td>Noise trauma (6 kHz for 15 min at 130 dB)</td>
<td>Behavior paradigm (Place-tone conditioning)</td>
<td>Tinnitus alleviation by ifenprodil</td>
<td>Gutton and Dudai (2007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td>Cochlea</td>
<td>Salicylate</td>
<td>Behavior paradigm (Active avoidance)</td>
<td>Activation of cochlear NMDA receptors</td>
<td>Gutton and others (2003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar rats</td>
<td>Inner hair cell Noise trauma (10 kHz for 2 hours at 120 dB)</td>
<td>Behavior paradigm (Activity in silence)</td>
<td>Reduction of inner hair cell ribbon loss by AM-101</td>
<td>Bing and others (2015)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMPA receptor</td>
<td>Human (22-83 years) Hearing loss Prebycusis Head trauma Labyrinthitis</td>
<td>Audiometry Questionnaire</td>
<td>Reduction of tinnitus severity by caroverine</td>
<td>Denk and others (1997)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA receptor</td>
<td>Human (24-58 years)</td>
<td>Audiometry Questionnaire</td>
<td>Reduction of tinnitus severity by gabapentin with lidocaine</td>
<td>Ciodaro and others (2015)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA level</td>
<td>Human (63 years) Stroke</td>
<td>Self-description</td>
<td>Effective improvement of tinnitus by low-doses Gabapentin</td>
<td>Chen and Yin 2012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (36-81 years)</td>
<td>Audometry Questionnaire</td>
<td>No significant improvement by gabapentin</td>
<td>Balshshee and others (2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (18-70 years)</td>
<td>Questionnaire</td>
<td>No significant improvement by gabapentin</td>
<td>Piccorillo and others (2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Class</th>
<th>Subclass</th>
<th>Subject</th>
<th>Region Tested</th>
<th>Tinnitus Induction</th>
<th>Type of Measure</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (29-84 years)</td>
<td></td>
<td></td>
<td></td>
<td>Questionnaire</td>
<td>No significant difference between placebo and patients treated with gabapentin</td>
<td>Witsell and others (2007)</td>
<td></td>
</tr>
<tr>
<td>Human (33-68 years)</td>
<td></td>
<td></td>
<td></td>
<td>Questionnaire</td>
<td>Partial effect in some tinnitus participants with gabapentin</td>
<td>Bauer and Brozoski (2006)</td>
<td></td>
</tr>
<tr>
<td>Human (35-82 years)</td>
<td></td>
<td></td>
<td></td>
<td>Pure tone audiometry</td>
<td>Tinnitus relief after acamprosate (86.9%)</td>
<td>Azevedo and Figueiredo (2005)</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td>Audimetry</td>
<td>Partial reduction by furosemide (2/14 patients)</td>
<td>Risey and others (1995)</td>
<td></td>
</tr>
<tr>
<td>Human (21-65 years)</td>
<td>Noise trauma</td>
<td></td>
<td></td>
<td>Questionnaire</td>
<td>76% reduction of tinnitus loudness with alprazolam</td>
<td>Johnson and others (1993)</td>
<td></td>
</tr>
<tr>
<td>CBA/CAj mice</td>
<td>Al</td>
<td>Noise trauma</td>
<td>16 kHz for 1 hour at 116 dB</td>
<td>Behavior paradigm</td>
<td>Decreased inhibition in tinnitus rats</td>
<td>Llano and others (2012)</td>
<td></td>
</tr>
<tr>
<td>SD rats</td>
<td>Al</td>
<td>Noise trauma</td>
<td>4 kHz for 7 hours at 123 dB</td>
<td>Behavior paradigm</td>
<td>Tinnitus alleviation by vigabatrin and NO711</td>
<td>Yang and others (2011)</td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td>MGB</td>
<td>Noise trauma</td>
<td>16 kHz for 1 hour at 116 dB</td>
<td>Behavior paradigm</td>
<td>Enhanced tonic inhibition in tinnitus rats</td>
<td>Sametsky and others (2015)</td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td>MGB</td>
<td>Noise trauma</td>
<td>16 kHz for 1 hour at 116 dB</td>
<td>Behavior paradigm</td>
<td>Decreased inhibition in tinnitus rats</td>
<td>Brozoski and others (2012)</td>
<td></td>
</tr>
<tr>
<td>CBA/CAj mice</td>
<td>DCN</td>
<td>Noise trauma</td>
<td>16 kHz for 45 min at 116 dB</td>
<td>Behavior paradigm</td>
<td>Decreased inhibition in tinnitus mice</td>
<td>Middleton and others (2011)</td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td></td>
<td>Noise trauma</td>
<td>16.3 kHz for 60 min at 120 dB</td>
<td>Behavior paradigm</td>
<td>Tinnitus behavior elimination by vigabatrin</td>
<td>Brozoski and others (2007)</td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td></td>
<td>Noise trauma</td>
<td>16 kHz for 60 min at 116 dB</td>
<td>Behavior paradigm</td>
<td>Tinnitus alleviation by taurine</td>
<td>Brozoski and others (2010)</td>
<td></td>
</tr>
<tr>
<td>Long-Evans rats</td>
<td></td>
<td>Noise trauma</td>
<td>16 kHz for 1, 2 hours at 105 dB</td>
<td>Behavior paradigm</td>
<td>Frequency-specific reversible alleviation by gabapentin</td>
<td>Bauer and Brozoski (2001)</td>
<td></td>
</tr>
<tr>
<td>Glycine receptor</td>
<td>Zinc</td>
<td>Human (60 years and older)</td>
<td>Questionnaire</td>
<td>Tinnitus treatment was ineffective by zinc</td>
<td>Coelho and others (2013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>Human (21-74 years)</td>
<td></td>
<td>Audiometry Match test Questionnaire</td>
<td>Tinnitus improvement in some patients by oral zinc medication</td>
<td>Arda and others (2003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class</td>
<td>Subclass</td>
<td>Subject</td>
<td>Region Tested</td>
<td>Tinnitus Induction</td>
<td>Type of Measure</td>
<td>Result</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>--------------------------</td>
<td>---------------</td>
<td>-------------------------------------------</td>
<td>----------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Zinc</td>
<td>Human</td>
<td>(19-67 years)</td>
<td>Acoustic trauma</td>
<td>Drug toxicity</td>
<td>Audiologic test, Questionnaire</td>
<td>Zinc supplement: Relief of tinnitus in some elderly patients with dietary zinc deficiency</td>
<td>Yetiser and others (2002)</td>
</tr>
<tr>
<td>GlyR</td>
<td>GF12</td>
<td>Long-Evans rats</td>
<td>Noise trauma (16 kHz for 90 min at 116 dB)</td>
<td>Behavior paradigm (Operant conditioned-suppression)</td>
<td>Tinnitus alleviation by taurine</td>
<td>Brozoski and others (2010)</td>
<td></td>
</tr>
<tr>
<td>GlyR α2</td>
<td>FBN rats</td>
<td>DCN</td>
<td>Noise trauma (17 kHz for 1 hour at 116 dB)</td>
<td>Behavior paradigm (Acoustic startle gap detection)</td>
<td>Decreased GlyR α2 levels</td>
<td>Wang and others (2009)</td>
<td></td>
</tr>
<tr>
<td>Na+ Channel</td>
<td>Human</td>
<td>(53 years)</td>
<td>Stroke</td>
<td>Self-description</td>
<td>Tinnitus suppression by Sodium Valproate</td>
<td>Menkes and Larson (1998)</td>
<td></td>
</tr>
<tr>
<td>K+ Channel</td>
<td>KCNE1</td>
<td>Human (~42 years)</td>
<td>Occupational noise</td>
<td>Questionnaire, Genotyping</td>
<td>Suggestive change of KCNE1 for tinnitus susceptibility</td>
<td>Pawelczyk and others (2012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KCNE3</td>
<td>Human (~50.1 years)</td>
<td></td>
<td>Audiometry, Questionnaire, Genotyping</td>
<td>No significant effects of KCNE3 on tinnitus susceptibility</td>
<td>Sand and others (2011)</td>
<td></td>
</tr>
<tr>
<td>KCNQ</td>
<td>ICR (CD-1) mice</td>
<td>DCN</td>
<td>Noise trauma (16 kHz for 45 min at 116 dB)</td>
<td>Behavior paradigm (Acoustic startle gap detection)</td>
<td>Prevention of tinnitus development by SF0034</td>
<td>Kalappa and others (2015)</td>
<td></td>
</tr>
<tr>
<td>BK channel</td>
<td>Albino SD rats</td>
<td>Salicylate</td>
<td></td>
<td>Behavior paradigm (SIPAC)</td>
<td>Dose-dependent alleviation of tinnitus by BMS-204352 Maxipost and its R-enantiomer</td>
<td>Lobaninas and others (2011)</td>
<td></td>
</tr>
<tr>
<td>HCN channel</td>
<td>ICR (CD-1) mice</td>
<td>DCN</td>
<td>Noise trauma (16 kHz for 45 min at 116 dB)</td>
<td>Behavior paradigm (SIPAC)</td>
<td>Reduction of HCN channel activity for tinnitus resilience</td>
<td>Li and others (2015)</td>
<td></td>
</tr>
</tbody>
</table>

NMDA receptor, N-methyl-D-aspartate receptor; AMPA receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GABA receptor, γ-aminobutyric acid receptor; BK channel, calcium ion-activated potassium channel; HCN channel, hyperpolarization-activated cyclic nucleotide-gated channel; KCNQ, slow delayed rectifiers of voltage-gated potassium channel; KCNE, voltage-gated potassium channel subfamily E. GlyR α1, glycine receptor subunit α1; D-AP5, D(-)-2-amino-5-phosphono pentanoic acid; AM-101, esketamine hydrochloride gel; AI, primary auditory cortex; MGB, medial geniculate body; DCN, dorsal cochlear nucleus; SIPAC, schedule-induced polydipsia avoidance conditioning; SD rats, Sprague–Dawley rats; FBN rats, Fischer Brown Norway rats; NIHl, noise induced hearing loss. Drug actions: neramexane, NMDA receptor antagonist; gacyclidine, noncompetitive NMDA receptor antagonist; memantine, NMDA receptor antagonist; flupirtine, NMDA antagonist; scarnposate, increases GABA transmission and reduces excitatory action of NMDA receptors; fenprofyll, NMDA antagonist; AM-101, noncompetitive small molecule NMDA receptor antagonist; gabapentin, a modulator of GABA biosynthesis-relating enzymes (i.e. glutamate decarboxylase, GAD, and branched chain amino transferase, BCAT); lidocaine, voltage-gated sodium channel antagonist; caroverine, AMPA receptor antagonist and noncompetitive NMDA receptor antagonist in high dose; Furosamide, a noncompetitive subtype-specific GABA A receptor (e.g., α6β2γ2) antagonist; alprazolam, GABA agonist; taurine, GABA and glycine receptor enhancer; Retigabine, KCNQ channel activator; SF0034, small-molecule activator synthesized from retigabine; BMS-204352 Maxipost, modulators of KCNQ and BK channels; R-enantiomer of Maxipost, modulators of KCNQ and BK channels; sodium valproate, antagonist of voltage-gated sodium/T-type calcium channels and enhancer of GABA level; vigabatrin, enhancer of GABA level; NO711, antagonist of GABA uptake; Zinc, a modulator of GlyRs.
It is important to note, however, that treatments using NMDA receptor antagonists have not always been successful in alleviating tinnitus symptoms (Figueiredo and others 2008; Yang and others 2011). Memantine, an open-channel NMDA receptor antagonist, failed to alleviate tinnitus of human participants (Figueiredo and others 2008). Also, memantine failed to alleviate salicylate-induced tinnitus in rats (Lobarinas and others 2006). The oral intake of flupirtine, a drug that antagonizes NMDA receptors by increasing Mg\(^{2+}\) blockage, could not treat tinnitus of participants (Salembier and others 2006). Moreover, patients suffered from severe side effects such as anamnesis and attention disorders; it is probably because of the global targeting of glutamate receptors in brain regions that are important for learning and memory such as hippocampus and prefrontal cortex. Nonetheless, many studies with humans and animals showed that antagonism of auditory NMDA receptors could be, in general, a strategy for treating tinnitus symptoms.

**γ-Aminobutyric Acid (GABA) Receptors**

Two types of major inhibitory receptors, ionotropic GABA\(_A\) and metabotropic GABA\(_B\) receptor, play a critical role in decreasing neuronal activity and maintaining homeostatic E/I balance in the brain (Wenner 2011). It is well known that neurotransmitter GABA plays an integral role in signal processing in auditory centers such as the cochlear nucleus, superior olivary complex, inferior colliculus, medial geniculate body (MGB), and auditory cortex (Brozoski and others 2012; Vater and others 1992). As such, altered inhibition has been implicated in hearing diseases including tinnitus.

Animals with noise-induced tinnitus have shown decreased GABAergic inhibition in the auditory DCN, thalamus, and cortex, suggesting the involvement of GABA metabolism in tinnitus (Brozoski and others 2012; Middleton and others 2011). In line with this, taurine, which is known as an enhancer of both GABA\(_A\) and GABA\(_B\) receptors, has been reported to ameliorate rat tinnitus behaviors following acoustic trauma (Brozoski and others 2010). Complementary to these findings, the reduction of GABAergic synaptic transmission in rat inferior colliculus was observed in salicylate-induced tinnitus models (Jastreboff 1995; Wang and others 2006). Furthermore, noise-induced tinnitus animals have showed reduction in GABA\(_A\)-mediated phasic and tonic inhibition along the auditory pathway (Brozoski and others 2012; Sametsky and others 2015; Yang and others 2011). Vigabatrin, a chemical that increases GABA levels, was used to successfully eliminate tinnitus behavior in noise-induced tinnitus rats (Brozoski and others 2007; Yang and others 2011). Gabapentin, a modulator of GABA biosynthesis-related enzymes (i.e., glutamic acid decarboxylase [GAD], and branched chain aminotransferase [BCAT]), has been used as tinnitus treatment in animals and patients (Bauer and Brozoski 2001; Chen and Yin 2012; Russell and Baloh 2009). However, one report revealed that thalamic GABAergic function was not altered in noise-induced tinnitus animals (Sametsky and others 2015).

As for clinical cases, alprazolam, a GABA\(_A\) receptor agonist, was able to decrease the loudness of tinnitus in 76% of participants compared with 5% of placebo group (Johnson and others, 1993). Intravenous administration of furosemide, a noncompetitive subtype-specific GABA\(_A\) receptor (e.g., \(\alpha_6\beta_2\gamma_2\)) antagonist, is reported to have reduced tinnitus severity in about 14% of tinnitus patients (Korpi and Luddens 1997; Risey and others 1995). Acamprosate, which is known to antagonize NMDA receptor-induced excitability, also acts on increasing GABA transmission. Its application to patients successfully relieved their tinnitus symptoms (Azevedo and Figueiredo 2005). In other clinical cases related with GABA transmission, it was found that gabapentin is largely ineffective in treating tinnitus (Bakhshaee and others 2008; Bauer and Brozoski 2006; Piccirillo and others 2007; Witsell and others 2007). When combined with intradermal administration of lido- caine, a sodium channel antagonist, however, gabapentin alleviated tinnitus symptoms up to about 6 months, compared with those that received gabapentin alone or placebo, suggesting that targeting more than one ion channel can be effective in tinnitus treatments (Ciòdaro and others 2015). So far, it is not clear how gabapentin can therapeutically help tinnitus due to its multivariate and unknown actions in neurons. Nonetheless, there is general consensus that augmenting GABA receptor-mediated inhibition is crucial to decrease hyperexcitability involved in tinnitus (Fig. 2).

**Glycine Receptors (GlyRs)**

GlyRs are known to gate chloride ions and mediate fast inhibitory neurotransmission mostly in the spinal cord, brainstem, and midbrain (Avila and others 2013; Betz 1992; Nicoll and others 1990; Probst and others 1986). However, the activity of GlyRs in the brain is also implicated in tinnitus.

Decreased GlyR α1 protein levels were observed in DCN of noise-induced tinnitus rats (Wang and others 2009). It is particularly of interest that a chemical, zinc ion, known to modulate GlyRs, could inhibit spontaneous firing of fusiform cells in mouse DCN (Perez-Rosello and others 2015). It should be noted that some studies suggested that zinc is ineffective except in some patients with zinc deficiency (Arda and others 2003; Coelho and others 2013; Person and others 2016; Yetiser and others 2002). Taurine, which activates both glycine and GABA
receptors, was used to palliate tinnitus behavior in rats; currently, it is not clear that the action of taurine on tinnitus is through GABA, glycine receptors or both (Brozoski and others 2010). Nonetheless, these results substantiate the notion that inhibition decrement is correlated with tinnitus. GlyRs, though being expressed mostly in lower brain areas, are believed to one key component for tinnitus development.

Figure 2. Homeostatic regulation of ion channels and receptors in hearing loss-induced tinnitus. (A) Hearing loss reduces γ-aminobutyric acid receptor (GABAR)–mediated phasic (Ai and Aii) and tonic (Aiii) inhibition, which is tightly related to tinnitus manifestation, as measured in miniature inhibitory postsynaptic currents and charge transfer, respectively. This figure is adapted and modified from Yang and others 2011. A schematic of synaptic homeostasis shows reduced inhibition, but not excitation which is presumably compensated from intercortical inputs. Note enlarged reddish color of a neuron indicates hyperexcitation in hearing loss-induced tinnitus. (B) Hearing loss increases intrinsic excitability by down-regulating IA channels (Bii and Biii). In our preliminary data, Kv1.4 (a A-type potassium channel) gene expression of the auditory cortex of tinnitus animals is down-regulated in both microarray and reverse transcription–polymerase chain reaction (rt-PCR) data. A schematic of intrinsic homeostasis shows exaggerated N-methyl-d-aspartate receptor (NMDAR) activity by down-regulation of IA channels in hearing loss-induced tinnitus (Biv).
Voltage-Gated Potassium Ion (K+) Channel

Voltage-gated K+ channels play a key role in dampening neuronal excitability and returning membrane potential to resting condition (Hille 2001). They have been reported to regulate neuronal excitability while their malfunction is involved in many types of brain diseases such as epilepsy, long-QT syndromes, ataxia/myokymia, Barter’s syndrome, neuromuscular disorders, hearing disorder, and vestibular diseases (Bernard and others, 2004; Shieh and others, 2000).

Mutation in KCNE1 (a voltage-gated potassium channel, Isk-related family member 1) was suggested to predispose humans to tinnitus development; however, such observation was not concrete because the finding could not be replicated in different patient populations (Pawelczyk and others 2012). Similarly, altered expression level of KCNNE3 in tinnitus patients could not reliably predict the risk of developing tinnitus due to small population size (Sand and others 2011). In noise-induced animal models, there is a decrease in KCNQ2/3 (a slow delayed rectifier of voltage-gated potassium channel) activity and accordingly an increase in neuronal activity of the DCN fusiform cells, both of which lead to increased susceptibility to tinnitus development (Li and others 2013). Consistent with this finding, retigabine, a small molecule that activates KCNQ channels and its variant (SF0034) prevented tinnitus development by suppressing noise-induced hyperexcitability in DCN neurons (Kalappa and others 2015). Application of retigabine reduced the membrane potential of DCN cells to more resting state, which conferred protection from developing tinnitus (Li and others 2015). A reduction in HCN channel activity was found in only one recent animal study (Li and others 2007; Yang and Cox 2008).

The involvement of HCN channels in tinnitus was observed in only one recent animal study (Li and others 2015). A reduction in HCN channel activity was found in the DCN of noise-induced tinnitus mouse. It is of interest that such reduction conferred resistance to tinnitus development. The result draws attention to the fact that HCN channels are involved in homeostatic compensation to hyperexcitability that would occur only after acoustic trauma. Further research into HCN channels is required to pinpoint their precise roles in processing intrinsic and extrinsic auditory signals.

Voltage-Gated Calcium Ion (Ca2+) Channels

Voltage-gated Ca2+ channels are localized to different compartments in a neuron and play different roles in regulating synaptic and firing activity. T- and L-type calcium channels are localized in dendrite and soma, while N- and P/Q-type calcium channels are at synaptic terminals (Dolphin 2012). It is also known that the T- and L-type calcium channels are responsible for subthreshold depolarization (or oscillations) and gene transcription whereas N- and P/Q-type calcium channels are critical for neurotransmitter release (Dolphin 2012; Hille 2001). Accordingly, dysfunction in these channels leads to salicylate-induced tinnitus behavior in rats; nonetheless, it is unclear whether the tinnitus alleviation was from the activation of KCNQ, BK channels or both (Hewawasam and others 2002; Lobarinas and others 2011). It is possible that BK channels contribute to restoration of depolarized neurons back to the resting state, thereby alleviating tinnitus. For now, further clinical validation is required.

Hypermolarization-Activated Cyclic Nucleotide-Gated Channels (HCN Channels)

HCN channels (or Ih, or other membrane family member 1) permit the passage of non-specific cations of Na+, Ca2+, and K+ and are abundantly expressed in most neurons (Benarroch 2013). Activated by hyperpolarization, HCN channels re-depolarize neurons, thereby bringing them close to threshold for spiking. They contribute to regulating neuronal excitability and synaptic transmission along the auditory pathway (Benarroch 2013; Kase and Imoto 2012; Kim and Holt 2013). It is also well-known that Ih enhances time-locking ability by shortening the cell’s time constant and reducing temporal width of spikes, eventually improving temporal precision of signals (Bal and Oertel 2000; Koch and Grothe 2003; Yang and Feng 2007; Yang and others 2009). Malfunction of these channels is involved in several brain diseases such as epilepsy, Parkinson’s disease, and neuropathic pain (Adams and others 2009; DiFrancesco and DiFrancesco 2015; Jung and others 2007; Yang and Cox 2008).

The involvement of HCN channels in tinnitus was observed in only one recent animal study (Li and others 2015). A reduction in HCN channel activity was found in the DCN of noise-induced tinnitus mouse. It is of interest that such reduction conferred resistance to tinnitus development. The result draws attention to the fact that HCN channels are involved in homeostatic compensation to hyperexcitability that would occur only after acoustic trauma. Further research into HCN channels is required to pinpoint their precise roles in processing intrinsic and extrinsic auditory signals.
various neurological disorders such as pain, epilepsy, ataxia, and psychiatric disorders (Frank 2014; Simms and Zamponi 2014).

Nimodipine, an L-type calcium channel antagonist, partially alleviated tinnitus of patients; improvement was observed in some patients, but worsening was in others after nimodipine treatment (Davies and others 1994; Elgoyhen and Langguth 2010). As described earlier (see GABA receptors), gabapentin, which is known to regulate GABA biosynthesis, had some positive effects on tinnitus alleviation. It is notable that the Gabapentin may also act via modulating calcium channels as it has been reported to bind to voltage-gated calcium ion channels and NMDA receptors, which are also calcium-permeable. It would be important to further investigate how different types of calcium channels regulate neuronal excitability and gene regulation of sound processing and tinnitus.

**Voltage-Gated Sodium Ion (Na+) Channel**

Voltage-gated Na⁺ channels are responsible for generation and propagation of action potentials in neurons (Catterall 2012). Because of this, they have usually been a major target of pharmacological therapy (Dib-Hajj and others 2015; Istvan and others 2007; Persson and others 2016; Zheng and Trudeau 2015).

Sodium valproate, a drug which blocks both voltage-gated sodium and T-type calcium channels, alleviated tinnitus symptoms in humans (Menkes and Larson 1998). However, another study reported that this drug triggered tinnitus during the treatment period (Hori and others 2003; Reeves and others 2009). Lidocaine, an antagonist of voltage-gated Na⁺ channels, has been also used for tinnitus research (Onizuka and others 2004). When administered intradermally to tinnitus patients, it caused substantial improvement in the loudness and level of tinnitus (Savastano 2004). Intradermal drug administration of lidocaine had less adverse effects than intravenous administration (Israel and others, 1982; Staffen and others 1999) and trans tympanical drug (Coles and others 1992) administration. However, the use of Na⁺ channel blockers for tinnitus is problematic because it disturbs normal cells’ excitability broadly throughout the brain. Further research on the dose-dependency of these drugs should be considered to avoid such a problem.

**Perspective**

Most drugs currently used for tinnitus treatment directly modulate ion channels and receptors (Table 1). However, the fact that those drugs act on a global scale over whole brain areas leads to discontinuation of pharmaceutical treatment due to nonspecific side effects (Langguth and others 2004; Salvi and others 2009). For example, drugs that serve as antagonists of NMDA receptors and voltage sodium channels for alleviating tinnitus can cause serious disruption of normal physiological functions along both auditory and nonauditory areas. Also, drugs that activate GABA receptors have emotionally sedating and even suicidal effects in tinnitus patients, leading to negative consequences on quality of life. One way to circumvent these problems is to consider methods of administration of therapeutic drugs locally. Local drug delivery to suppress tinnitus could lead to better clinical outcomes, avoiding aberrant brain activity in different regions. Additionally, the location and mechanisms of tinnitus could be readily identified if local drug delivery is available. A second consideration is to identify optimized drug concentration. An optimized concentration can be effective only in the affected auditory areas even when administered globally in the brain. Last, it would be beneficial to apply a drug that indirectly modulates ion channels/receptors which are essential for normal excitability of neurons (e.g., Na⁺ channels, NMDA and GABA receptors). Our group previously found that noise-induced tinnitus animals exhibit intrinsic hyperexcitability (Yang and others 2012). If tinnitus is related to exaggerated activity of NMDA receptors, it would be beneficial for tinnitus alleviation to employ an enhancer of A-type potassium channels (or Iₐ). It is because Iₐ is known to be activated largely in a hyperexcitable condition of NMDA receptors, both of which have been known to play a counterbalancing role in regulating neuronal excitability (Yang and others 2014; Yang and others 2015). Furthermore, Iₐ was, in our preliminary data, downregulated in tinnitus animals and accordingly its activation can potentially alleviate tinnitus by suppressing intrinsic hyperexcitability, but does not severely affect normal signal transmission and membrane excitability (Fig. 2). Thus, the indirect modulators of physiologically essential channels/receptors should be considered to design therapeutic target of tinnitus. As mentioned above (Glutamate Receptors section), drugs that can indirectly modulate the target receptors should also be considered as viable treatments of tinnitus. Those strategies might not interfere with the normal physiological function of individuals, therefore having long-term clinical benefits.

Tinnitus, such as the condition caused by hearing loss, induces widespread changes in expression and function of ion channels and receptors along the auditory pathway. These changes can be the primary effect of the hearing loss, or a secondary effect caused by homeostatic compensation executed by the various neurons in the system. Gene expression of ion channels is a potent way to modify the membrane composition of ion channels and receptors in a given neuron, and hence its excitability and E/I balance (Fig. 3). Many molecules are involved in the genetic regulation of ion channels and receptors:
transcription factors of the immediate-early gene Arc/Arg3.1, secreted factors such as retinoids, TGF-β, TNF-α, and BDNF, and signaling molecules such as CaMKIIα and β, TGF-β, TNF-α, and BDNF) to be shifted into increased E/I balance. NMDAR, N-methyl-D-aspartate receptor; AMPAR, AMPA receptor; VGCC, voltage-gated calcium channel; VGSC, voltage-gated sodium channel; HCN, Hyperpolarization-activated cyclic nucleotide-gated channel; GABAR, GABA receptor; GlyR, glycine receptor; KCNE, voltage-gated potassium channel subfamily E; KCNQ, slow delayed rectifiers of voltage-gated potassium channel; I_A, A-type voltage-gated potassium channels; BK, calcium ion activated potassium channel.

Figure 3. A seesaw model of tinnitus channelopathy. Environmental (e.g., auditory trauma) and genetic (e.g., overexpression of genes susceptible to tinnitus) changes cause action of homeostatic molecules (retinoids, Arc/Arg3.1, CaMKIIα and β, TGF-β, TNF-α, and BDNF) to be shifted into increased E/I balance. NMDAR, N-methyl-D-aspartate receptor; AMPAR, AMPA receptor; VGCC, voltage-gated calcium channel; VGSC, voltage-gated sodium channel; HCN, Hyperpolarization-activated cyclic nucleotide-gated channel; GABAR, GABA receptor; GlyR, glycine receptor; KCNE, voltage-gated potassium channel subfamily E; KCNQ, slow delayed rectifiers of voltage-gated potassium channel; I_A, A-type voltage-gated potassium channels; BK, calcium ion activated potassium channel.


