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Metabotropic glutamate receptors in auditory processing

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Abstract

As the major excitatory neurotransmitter used in the vertebrate brain, glutamate activates ionotropic and metabotropic glutamate receptors (mGluRs), which mediate fast and slow neuronal actions, respectively. Important modulatory roles of mGluRs have been shown in many brain areas, and drugs targeting mGluRs have been developed for treatment of brain disorders. Here, I review the studies on mGluRs in the auditory system. Anatomical expression of mGluRs in the cochlear nucleus has been well characterized, while data for other auditory nuclei await more systematic investigations at both the light and electron microscopy levels. The physiology of mGluRs has been extensively studied using *in vitro* brain slice preparations, with a focus on the lower auditory brainstem in both mammals and birds. These *in vitro* physiological studies have revealed that mGluRs participate in neurotransmission, regulate ionic homeostasis, induce synaptic plasticity, and maintain the balance between excitation and inhibition in a variety of auditory structures. However, very few *in vivo* physiological studies on mGluRs in auditory processing have been undertaken at the systems level. Many questions regarding the essential roles of mGluRs in auditory processing still remain unanswered and more rigorous basic research is warranted.

Keywords

mGluR; auditory processing; neurotransmission; neuromodulation; excitotoxicity; synaptic plasticity

INTRODUCTION

Glutamate is the most abundant excitatory neurotransmitter in the vertebrate brain. Upon release at synapses glutamate activates ionotropic receptor channels and metabotropic glutamate receptors (mGluRs), which mediate fast and slow neuronal actions, respectively. Since the discovery of mGluRs nearly thirty years ago (Sladeczek et al., 1985; Nicoletti et al., 1986a, 1986b), eight members of mGluRs have been identified. They have been divided

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into three groups (group I: mGluR1 and 5; group II: mGluR2 and 3; and group III: mGluR4, 6, 7, and 8) based on their amino acid sequence, pharmacological properties, and signaling transduction pathways (reviewed in Niswender and Conn, 2010). mGluRs are expressed throughout the peripheral and central nervous system, exhibit a high degree of homology across different animal species, and exert neuromodulatory actions via multiple signaling pathways (reviewed in Ferraguti and Shigemoto, 2006; Nicoletti et al., 2011; Tharmalingam et al., 2012). Group I mGluRs are predominantly expressed at postsynaptic loci and are coupled primarily to G_q/G_{11} proteins associated with stimulation of the phospholipase C pathway. Group II and III mGluRs are predominantly expressed at presynaptic loci and are coupled to G_i/G_o proteins associated with inhibition of the adenylyl cyclase pathway. Because of their important modulatory roles under physiological as well as pathological conditions, mGluRs have been implicated in multiple brain disorders (reviewed in Swanson et al., 2005; Krystal et al., 2010), and drugs targeting mGluRs have been on clinical trials for treatment of schizophrenia (Patil et al., 2007) and autism (Oberman, 2012). While this review is focused on mGluRs in the auditory system, readers are referred to several excellent reviews on the general topics of mGluRs (Cartmell and Schoepp, 2000; Pinheiro and Mulle, 2008; Olive, 2009; Krystal et al., 2010; Niswender and Conn, 2010; Nicoletti et al., 2011; Lodge et al., 2013).

At all levels of the auditory system, it is conceivable that mGluRs are involved in information processing, considering that glutamate is used as the major excitatory neurotransmitter from the peripheral hearing organ (the cochlea) all the way up to the auditory cortex (AC), and that mGluRs have been found to be expressed in a variety of auditory structures. Understanding the anatomy and physiology of mGluRs at various levels of the auditory system will not only provide an in-depth understanding of mechanisms that underlie auditory processing, but may also help design potential therapeutic approaches targeting mGluRs for treatment of hearing disorders. Here, I will first review the anatomical expression and physiology of mGluRs in the mammalian auditory system, with a focus on the cochlea, a number of nuclei in the lower auditory brainstem, the auditory midbrain, the auditory thalamus, and the AC. Then, I will review the studies of mGluRs in the avian lower auditory brainstem, and propose directions for future studies.

OVERVIEW OF ANATOMICAL EXPRESSION OF mGluRs IN THE MAMMALIAN AUDITORY SYSTEM AND CONSIDERATION OF PHYSIOLOGICAL METHODS

Anatomical data on the expression of mGluRs in the mammalian auditory system are summarized in Table 1. The data are extracted primarily from two sources. First, data are available from anatomical studies where the expression of mGluRs is examined throughout the whole brain (e.g., Shigemoto et al., 1992; Ohishi et al., 1993a, 1993b, 1995b, 1998; Bradley et al., 1998). Because the auditory system is not the focus of these studies, the data on mGluR expression in the auditory system lack details. An exception to this lack of details may be the data on the expression of mGluRs in the cochlear nucleus (CN), which has been studied more in-depth than any other auditory structure (Ohishi et al., 1995, 1998; Wright et al. 1996; Petralia et al., 1996a, 1997; Bilak and Morest, 1998; Bradley et al., 1998;

Kinoshita et al., 1998; Kemmer and Vater, 2001; Irie et al., 2006; Dino and Mugnaini, 2008). Second, data on the expression of mGluRs in the auditory system are available from physiological studies where anatomical data were obtained to support the physiology (e.g., Kushmerick et al., 2004; Nishimaki et al., 2007; Lee and Sherman, 2012), but generally, these studies did not provide in-depth details about the anatomy of mGluRs. Overall, the anatomical data of mGluRs in the auditory system are far from being complete. However, one general impression is that mGluRs are extensively expressed in cells at various levels of the auditory system. In order to understand the roles of mGluRs in auditory processing one key future direction is to systematically investigate the expression of mGluRs in the auditory system, both at the light and electron microscopy levels.

Most physiological studies on mGluRs in the central auditory system have been performed using *in vitro* brain slice preparations. The use of *in vitro* brain slices allows for stable and reliable intracellular whole-cell recordings for long periods of time (Dingledine et al., 1980; Trussell, 1999), making possible studies of both short- and long-term effects of mGluRs on neuronal properties. This approach also allows for application of pharmacological agents at known concentrations. Precise control of the concentration of pharmacological agents is especially critical for studying the effects of mGluR agonists and antagonists, considering that subtype or group member specificity of drugs can be concentration specific (reviewed by Nicoletti et al., 2011). The physiological results reviewed below are obtained using whole-cell recordings in brain slices, unless indicated otherwise. It is worthwhile to mention an advanced patch recording method, perforated patch recording. Because perforated patch recording can better preserve the intracellular signaling molecules (Horn and Marty, 1988; Kyzozis and Reichling, 1995; Fan and Palade, 1998), it is particularly useful when examining the effect of mGluRs in postsynaptic cells. It is known that under whole-cell recording mode, one could potentially wash out intracellular signaling molecules required for G-protein-coupled receptors to function (Trussell and Jackson, 1987; Vargas et al., 1999; Thomas et al., 2004). This would lead to disruption of the signaling pathway and consequently observation of false-negative results. Supporting this notion, we recently observed that activation of mGluRs enhanced voltage-gated K^+ channel currents in timing coding neurons in the auditory brainstem, and the modulation was detected under perforated patch recording but not under conventional whole-cell recording (our unpublished data). This points to the need and importance of using perforated patch recording to study mGluR effect on postsynaptic properties, especially when one observes negative results by using conventional whole-cell recordings.

ANATOMY AND PHYSIOLOGY OF mGluRs IN THE MAMMALIAN COCHLEA

Expression of mGluRs in the cochlea

Glutamate is the major excitatory transmitter used at the synapses between hair cells and spiral ganglion neurons, and both ionotropic and metabotropic glutamate receptors are expressed in the cochlea (reviewed in Eybalin, 1993; Niedzielski et al., 1997). Of the group I mGluRs, messenger RNA (mRNA) for mGluR1 has been detected in both type I and type II neurons in cochlear ganglion cells of the rat and guinea pig (Safieddine and Eybalin, 1995). Anatomical data on group II mGluRs in the cochlea are lacking, although a physiological

study suggested that group II mGluRs are expressed on the efferent lateral olivocochlear GABAergic fibers in the guinea pig (Doleviczényi et al., 2005). Of the group III mGluRs, mGluR7, is expressed in both inner and outer hair cells (IHCs and OHCs), as well as in spiral ganglion cells in mouse and human tissues (Friedman et al., 2009). The expression of mGluR7 in the mouse cochlea exists at birth (postnatal day 1, P1) and persists into maturation (P21 and adult) (Friedman et al., 2009).

Physiology of mGluRs in the cochlea

Of the mGluRs shown to be expressed in the cochlea, physiological evidence for involvement of mGluR1 (group I) and mGluR7 (group III) in neurotransmission at the synapses between hair cells and the dendrites of spiral ganglion neurons has been reported. Group II mGluRs are involved in modulation of neurotransmission at the lateral olivocochlear efferent system (Fig. 1A).

Physiology of group I mGluRs in the cochlea

An excitatory action mediated by group I mGluRs in the cochlea has been demonstrated. Application of DHPG, an agonist for group I mGluRs, increases the spike firing of spiral ganglion neurons (Kleinlogel et al., 1999; Oestreicher et al., 2002), accompanied by an increase in intracellular Ca^{2+} concentration (Peng et al., 2004). In spiral ganglion neurons ACPD, a non-specific mGluR agonist that predominantly activate group I and II mGluRs, produces an inward current under voltage clamp, and causes membrane depolarization and spiking under current clamp (Peng et al., 2004). These studies indicate that group I mGluRs are located postsynaptically on the IHC afferents of spiral ganglion neurons. The excitatory effects of group I mGluRs in the cochlea generally last longer than those mediated by ionotropic glutamate receptors. This suggests that mGluRs do not mediate the fast neurotransmission in the cochlea where a high speed of transmission is essential for transduction of acoustic signals, but instead may be more important for enhancing the cellular excitability of spiral ganglion neurons under high frequency inputs.

Physiology of group II mGluRs in the cochlea

Modulation rather than mediation of synaptic transmission by group II mGluRs has been reported in the cochlea. *In vitro* microvolume superfusion on guinea pig cochlea preparations shows that pharmacological activation of group II mGluRs, but not group I and III mGluRs, increases dopamine release at the dopaminergic synapses of the lateral olivocochlear system (Doleviczényi et al., 2005). Increased dopamine can activate D_2 receptors on IHC afferents, and may lead to protective effects against excitotoxicity caused by excessive glutamate release from the IHCs in response to noise exposure. It is believed that the mechanism for increased dopamine release is via a sequence of modulatory actions at multiple synapses, including activation of group II mGluRs on GABAergic terminals, which suppresses GABA release, leading to disinhibition of dopaminergic terminals innervating IHC afferents (Doleviczényi et al., 2005). Given the complexity of this hypothetical pathway, physiological recordings such as patch recordings from the IHC afferents are needed to confirm the direct and indirect actions of each transmitter involved.

Physiology of group III mGluRs in the cochlea

Physiological data on group III mGluRs in the cochlea are largely lacking, although there is evidence for the involvement of mGluR7 variants in conferring susceptibility to age-related hearing loss (Friedman et al., 2009). Because mGluR7 is expressed at both pre- and postsynaptic loci at the synapses between hair cells and ganglion neurons (Friedman et al., 2009), the protein may provide protection against glutamate excitotoxicity via suppression of glutamatergic transmission in the cochlea.

Because group I and the other two groups of mGluRs have opposite effects on excitatory transmission at IHC synapses, one would expect that antagonists for group I may provide protection against excitotoxicity, while agonists for group II or III mGluRs may have the same effect. The idea of using antagonism for one group of mGluRs and agonism for other groups of mGluRs to achieve the same purpose reflects the complexity of mGluR-mediated actions. Indeed, even for the same group of mGluRs, there seem to be conflicting proposals regarding whether and how they provide protection of the cochlea against acoustic damage. Protective effects of mGluR1 against pathological conditions were proposed based on the observation that mGluR1 level was up regulated in response to excitotoxic insults (Puel et al., 1995). In contrast, blocking group I mGluRs in the cochlea reduces the amplitude of compound action potentials in response to loud sound without altering the hearing threshold (Peng et al., 2004). This suggests that antagonism, rather than increased activity of group I mGluRs may provide protective effects to the cochlea.

ANATOMY AND PHYSIOLOGY OF mGluRs IN MAMMALIAN COCHLEAR NUCLEUS

Expression of mGluRs in the CN

Among the auditory structures, the CN, especially the dorsal cochlear nucleus (DCN, a cerebellum-like structure, Mugnaini et al., 1980), is the most extensively studied in terms of mGluR expression (previously reviewed in Petralia et al., 2000).

Expression of group I mGluRs in the CN

Of the two group I subtypes, there is greater amount of data for expression of mGluR1 than for mGluR5 in the CN. The expression of mGluR1 in the CN has been studied in several animal species including mice (Bilak and Morest, 1998), rats (Petralia et al., 1996a, 1997), guinea pigs (Wright et al., 1996), and bats (Kemmer and Vater, 2001). At both the mRNA and protein levels, expression of mGluR1 is reported to vary not only between different divisions of the CN, but between different cell types within the same division (Petralia et al., 1996a; Wright et al., 1996; Bilak and Morest, 1998). The overall expression level of mGluR1 is strongest in the DCN, moderate in the anteroventral CN (AVCN), and weak in the posteroventral CN (PVCN) (mice: Bilak and Morest, 1998; rats: Shigemoto et al., 1992; Petralia et al., 1997; bats: Kemmer and Vater, 2001). While most cell types in the rodent DCN, including unipolar brush cells and cartwheel cells, strongly express mGluR1 (Wright et al., 1996; Dino and Mugnaini, 2008), granule cells in the molecular and fusiform cell layers do not express this protein (Bilak and Morest, 1998). mGluR1 is most commonly

observed in postsynaptic compartments of DCN neurons (Petrulia et al., 1996a; Wright et al., 1996). However, it is also detected on terminal axons in the molecular layer and small cell shell of the DCN (Bilak and Morest, 1998), suggesting presynaptic actions mediated by mGluR1. In the ventral CN (VCN), a high level of mGluR1 is found in globular bushy cells and stellate cells (Bilak and Morest, 1998). The expression of mGluR5 is weak or non-detectable in the DCN (Petrulia et al., 1996a), and its expression in the VCN is not known.

Expression of group II mGluRs in the CN

In a study that investigated mGluR2 expression in the CNS of the rat and mouse (Ohishi et al., 1998), mGluR2 was found to be expressed in the DCN, with strong expression in the granular layer. The cell types expressing mGluR2 in the DCN seem to be the unipolar brush cells and Golgi cells (Petrulia et al., 1996b; Irie et al., 2006), similar to the cell types that express mGluR2 in the cerebellum (Ohishi et al., 1998). The mGluR2 immunoreactivity was also observed in the VCN in sparsely scattered cells (Ohishi et al., 1998), which are possibly stellate cells with thin dendrites. In addition, expression of mGluR2 was observed in both the soma-dendritic domain and the axonal domain in the DCN (Petrulia et al., 1996b; Ohishi et al., 1998), suggesting that mGluR2 may exert both pre- and postsynaptic modulation on CN neurons. No data are available for the expression of mGluR3 in the CN.

Expression of group III mGluRs in the CN

Compared to group I and II mGluRs, much less data are available about the expression of group III mGluRs in the CN, of which there are four members (mGluR4, 6, 7, and 8). This observation is generally true for the entire auditory system (Table 1). A low level of mGluR4 mRNA and a moderate level of mGluR7 mRNA exist in the CN of the rat (Ohishi et al., 1995). Among the few studies that investigated protein expression of mGluR7 in the CN, results are not consistent. Bradley et al. (1998) reported positive expression of mGluR7 in the DCN of the rat, whereas Kinoshita et al. (1998) did not detect immunoreactive labeling in the same animal species. Because these studies examine the expression of mGluRs in the whole brain without a focus on the auditory system, firm conclusions on the expression of group III mGluRs in the CN cannot yet be drawn.

Physiology of mGluRs in the CN

While a relatively large number of studies have examined the expression of mGluRs in the CN (Table 1), only a few physiological studies have reported the functional roles of mGluRs using *in vitro* brain slice preparations (Molitor and Manis, 1997; Fujino and Oertel, 2003; Irie et al., 2006; Chanda and Xu-Friedman, 2011) (Fig. 1B). In the DCN, one physiological study used field potential recordings in guinea pig brain slices, and found that all three groups of mGluRs appear to depress the field responses evoked by electrical stimulation of the parallel fibers to the DCN (Molitor and Manis, 1997). Long-term plasticity involving mGluRs was observed in the mouse DCN (Fujino and Oertel, 2003). Multiple postsynaptic mGluRs, possibly group II and III mGluRs, contribute to the induction of both long-term potentiation (LTP) and long-term depression (LTD) of excitatory postsynaptic currents (EPSCs) in fusiform and cartwheel cells in the DCN. Interestingly, such long-term plasticity is afferent input-specific. The plasticity is induced for the parallel fiber multisensory input, but not the auditory fiber input (Fujino and Oertel, 2003). Contributions of mGluRs to such a

bidirectional synaptic plasticity (both LTP and LTD) in the same neurons indicate possible roles of mGluRs in auditory learning. In Golgi cells in the DCN, postsynaptic group II mGluRs generate a membrane hyperpolarization, which is mediated by the activation of G-protein-coupled inward rectifier K⁺ (GIRK) channels (Irie et al., 2006).

In the VCN, even though the expression of mGluR5 is not known and its expression in DCN is weak (Petrálie et al. 1996a), pharmacological activation of group I mGluRs (primarily mGluR5) depolarizes bushy cells in the AVCN, and more importantly tonic activity of group I mGluRs is present (Chanda and Xu-Friedman, 2011). It is thus proposed that group I mGluRs facilitate the excitability of bushy cells, counteracting presynaptic inhibition mediated by GABA_B receptors (GABA_BRs) (Chanda and Xu-Friedman, 2011).

Studies using *in vivo* recording approaches to examine the physiological roles of mGluRs in auditory processing are few, in the CN in particular and in the entire auditory system in general. To date, for the CN, there is only one *in vivo* study on mGluRs done in the cat and gerbil at the systems level (Sanes et al., 1998). Bidirectional modulation of neuronal firing in the DCN by iontophoretic application of generic mGluR agonists (ACPD or CCG) was observed (Sanes et al., 1998). The varying effects of mGluRs on the auditory responses of CN neurons can be attributed to the presence of multiple subtypes of mGluRs on multiple loci of different cell types. Further studies combining mGluR drugs that target specific mGluR members and recordings from identified cell types should be performed. The difficulty of distinguishing the anatomically intermingled multiple cell types in the CN may be reduced dramatically with the development of new tools, such as an optogenetic approach that allows activation of specific cell types using light stimuli. Alternatively, the avian CN provides a more feasible model in this regard as compared to the mammalian system, because of the relatively high homogeneity of cell types and the ease of identification of cells in slice preparations (see below).

ANATOMY AND PHYSIOLOGY OF mGluRs IN MAMMALIAN MNTB

Expression of mGluRs in the medial nucleus of trapzoid body (MNTB)

Expression of mGluRs in the MNTB has been reported, with varying receptor locations depending on the subtype of mGluRs. The expression of mGluR1 is found to be exclusively postsynaptic in MNTB neurons in the rat (Kushmerick et al., 2004). These receptors are presumably located on the postsynaptic membrane that is opposite to presynaptic cannabinoid type 1 receptors (Kushmerick et al., 2004). Group II mGluRs are expressed at both presynaptic and postsynaptic domains of MNTB neurons in the rat, detected by using an antibody against both members (mGluR2/3) (Elezgarai et al., 2001). Group II mGluRs are also present on glial cells during postnatal development and diminish after maturation (Elezgarai et al., 2001). One member of group III mGluRs, mGluR4, is expressed at the presynaptic glutamatergic terminals innervating MNTB principle neurons in the rat. The expression is highly developmentally regulated, with strong activity in a short period (P7-12) before hearing onset (at around P13) (Elezgarai et al., 1999). These results suggest that group II mGluRs and the group III mGluR4 may be involved in the development of synapses in the MNTB. Whether group I mGluRs in the MNTB experience similar developmental change is not known.

Physiology of mGluRs in the MNTB

Activation of postsynaptic mGluR1 in MNTB neurons leads to release of endocannabinoids, which activate type 1 cannabinoid receptors on the presynaptic terminal (calyx of Held), resulting in inhibition of Ca^{2+} channels and subsequent suppression of glutamatergic transmission (Kushmerick et al., 2004) (Fig. 1C). Besides mediating such retro-suppression of neurotransmission, activation of group I mGluRs also increases phosphorylation of $\text{K}_v3.1b$ (Song and Kaczmarek, 2006), which is a critical factor defining the ability of MNTB neurons to follow high frequency inputs (reviewed in Johnston et al., 2010). Physiological function of group II mGluRs in MNTB is not known. However, because group II mGluRs are expressed at both pre- and postsynaptic loci in MNTB (Elezgarai et al., 2001), it is conceivable that modulation of glutamatergic transmission via actions on both sites exists. Activation of group III mGluRs by agonists has been shown to reduce EPSC amplitude in MNTB neurons (Billups et al., 2005). Interestingly, blocking the endogenous activity of group III mGluRs with antagonists does not modulate synaptic strength (EPSC amplitude). Rather, it lowers release probability and increases the size of the readily releasable pool (Billups et al., 2005). This raises an intriguing possibility that, in the auditory brainstem, endogenous activity of mGluRs may change the synaptic state even when modulation of synaptic strength by antagonism of mGluRs is not observed.

ANATOMY AND PHYSIOLOGY OF mGluRs IN MAMMALIAN LSO

Expression of mGluRs in the lateral superior olive (LSO)

In the LSO, group II mGluRs (mGluR2/3) are expressed at an early developmental age (P4), and the expression diminishes days after hearing onset in the rat (Nishimaki et al., 2007). The loci of group II mGluRs in the LSO are not known, although physiological data suggest presynaptic expression at both glutamatergic and glycinergic terminals (Wu and Fu, 1998; Nishimaki et al., 2007), as well as postsynaptic loci (Kotak and Sanes, 1995; Ene et al., 2003).

Physiology of mGluRs in the LSO

Consistent with the observation that the anatomical expression of mGluRs peaks during development and diminishes after maturation (Nishimaki et al., 2007), effects of mGluRs in the LSO have been detected mainly in developing neurons (Fig. 1C). The non-specific mGluR agonist ACPD depolarizes LSO neurons in P8-14 gerbils, and the effect is long-lasting (~20 min) (Kotak and Sanes, 1995). Calcium imaging studies show that activation of group I and II, but not III, mGluRs causes increases in intracellular Ca^{2+} concentration in developing LSO neurons (P0-4) (Ene et al., 2003), and the response amplitude reduces over the period of hearing development (from P0 to P20) (Ene et al., 2007). The group I mGluR-mediated Ca^{2+} signaling is partially due to Ca^{2+} influx through transient receptor potential-like channels, which can be activated by mGluR1 (Kim et al., 2003). The effects of mGluRs observed in these studies (Kotak and Sanes, 1995; Ene et al., 2003, 2007) are attributed to activation of postsynaptic mGluRs, because the parameters measured are properties directly obtained from postsynaptic cells.

Modulatory effects of presynaptic mGluRs at LSO synapses have been also reported (Fig. 1C). The non-specific mGluR agonist ACPD inhibits excitatory postsynaptic potentials (EPSPs) of LSO neurons in P14-22 rats, and at least group III mGluRs are involved (Wu and Fu, 1998). Presynaptic modulation by group II mGluRs of the inhibitory glycinergic transmission at the MNTB-LSO synapse has been also reported (Nishimaki et al., 2007). It is proposed that glutamate released from VCN neurons at the LSO spills over and activates mGluRs on MNTB presynaptic terminals innervating LSO neurons, producing heterosynaptic modulation. This modulation diminishes gradually in early development and is not detectable a few days after hearing onset (Nishimaki et al., 2007), which suggests that mGluRs may play a role in the development of this synapse.

ANATOMY AND PHYSIOLOGY OF mGluRs IN MAMMALIAN AUDITORY MIDBRAIN

Expression of mGluRs in the inferior colliculus (IC)

An RT-PCR method detected mRNA for group I mGluRs (mGluR1, 5) in the rat IC, and the expression level is developmentally down regulated (Martinez-Galan et al., 2012). In addition, all members of group III mGluRs (mGluR4, 6, 7, and 8) are expressed in the rat IC (Yip et al., 2001). An unusual observation is the detection of mGluR6 in IC tissues (Yip et al., 2001). It is unusual because mGluR6 is believed to be expressed exclusively in the retina (Nakajima et al., 1993). However, expression of mGluR6 in non-retinal neural tissues has also been reported (Faden et al., 1997; Ghosh et al., 1997). Given these conflicting results, the observation of mGluR6 in the IC should be firmly substantiated in future studies. The IC is a hub of the auditory system, which receives ascending and descending inputs from many auditory nuclei. Consequently, the IC is divided into multiple sub-nuclei, which contain multiple cell types with distinct anatomy and physiology. Based on the currently available data, it is largely unknown which areas and cell types in the IC express which mGluRs.

Physiology of mGluRs in the IC

A few studies have examined mGluR-mediated modulation in the IC, with a focus on the central part of the nucleus (Fig. 1D). Blocking mGluRs with a non-specific antagonist MCPG increases the gain in action potential firing of IC neurons (Miko and Sanes, 2009). Antagonism but not activation of group II mGluRs also increases firing of IC neurons *in vivo* (Voytenko and Galazyuk, 2011), suggesting suppression of cellular excitability by endogenous activity of group II mGluRs. Using whole-cell patch recordings in brains slices, Farazifard and Wu (2010) showed that activation of group II mGluRs suppresses both EPSCs and inhibitory postsynaptic currents (IPSCs) in IC neurons, and the effects are mediated by presynaptic actions. In contrast, activation of group I or III mGluRs modulates neither the synaptic responses nor the intrinsic properties (Farazifard and Wu, 2010). However, the negative results on group I mGluRs in this study seem to conflict with those reported by other research groups. For example, activation of group I mGluRs results in increase of intracellular Ca^{2+} concentration in IC neurons (Martinez-Galan et al., 2012). Furthermore, iontophoretic application of group I mGluR agonists increases firing rate of IC neurons *in vivo* (Voytenko and Galazyuk, 2011). However, because iontophoretic application

does not allow for precise estimate of the drug concentration (Stone, 1985), alternative techniques that apply drugs with known concentration such as pressure ejection may be considered in future studies. Finally, anticonvulsant actions of mGluRs, especially by group III mGluRs in genetically epilepsy-prone animals have been reported (Tang et al., 1997; Yip et al., 2001), raising the possibility of drugs targeting mGluRs for treatment of audiogenic seizures.

PHYSIOLOGY OF mGluRs IN MAMMALIAN AUDITORY THALAMUS

Expression of mGluRs in the medial geniculate body (MGB)

The very limited data on mGluR expression in the MGB come from a few papers that studied mGluR expression in the rat brain. Protein expression of the group I mGluR1 was detected in the thalamus including the MGB, and the expression loci seem to be at postsynaptic cells only (Martin et al., 1992; Fotuhi et al., 1993). Group II mGluRs are also expressed in the MGB, as detected with an antibody against mGluR2/3 (Petrálie et al., 1996b).

Physiology of mGluRs in the MGB

Very few studies have examined the physiology of mGluRs in the MGB. Activation of mGluRs by ACPD causes a depolarizing effect (inward current, or membrane depolarization) in MGB neurons recorded in slice preparations (Tennigkeit et al., 1999; Schwarz et al., 2000). The effect may be mediated via the descending input from the AC, but not from the ascending input from the IC because mGluR-mediated responses in the thalamus were observed via the descending glutamatergic input only (McCormick and von Krosigk, 1992; Eaton and Salt, 1996). Because ACPD is a non-specific mGluR agonist, it is unclear which mGluRs mediate the response. However, the authors suggested that mGluR1 and mGluR2 may be involved based on the concentration of ACPD (50 μ M) and its EC₅₀ on mGluRs (105–170 μ M for mGluR1, and 5 μ M for mGluR2) (Tennigkeit et al., 1999) (Fig. 1E). It is worth noting that while the ascending input from the IC via the lemniscal pathway to MGB neurons does not activate mGluRs, the ascending input from the IC via the non-lemniscal pathway to the dorsal part of the auditory thalamus does involve a mGluR1 component (Lee and Sherman, 2010). Further works need to be done in order to examine if and how mGluRs modulate synaptic transmission in the auditory thalamus.

PHYSIOLOGY OF mGluRs IN MAMMALIAN AUDITORY CORTEX

Expression of mGluRs in the AC

At the light microscopy level, group II mGluRs (mGluR2/3) are expressed in various neocortical regions including the AC, and these mGluRs co-localize with vesicular glutamate transporter 2 (Lee and Sherman, 2009a, 2012). This suggests the presence of presynaptic group II mGluRs on the excitatory terminals of axons that project into the AC.

Physiology of mGluRs in the AC

The AC constitutes a number of structurally distinct layers, and there exist multiple cell types in a single layer. Neurons in the AC receive ascending inputs from the auditory

thalamus, and form intracortical circuits among different layers. Involvement of mGluRs in neuromodulation in the AC is dependent on the input pathways (Fig. 2).

Physiology of mGluRs in layers 2 and 3 of the AC

Bidirectional regulation by mGluRs of synaptic transmission and cellular excitability of AC neurons has been reported. In pyramidal neurons in layers 2 and 3, presynaptic mGluRs activated by non-specific agonist ACPD inhibit both EPSPs and inhibitory postsynaptic potentials (IPSPs) elicited by stimulation of the synaptic inputs originating from layer 6 of the AC. In fast spiking neurons postsynaptic group I mGluRs increase cellular excitability (Bandrowski et al., 2001, 2002). The involvement of mGluR component in the excitatory responses of pyramidal cells is cell class-dependent, in that a group I mGluR component exists in class 2 cells (modulator neurons) but not in class 1 cells (driver neurons) (Viaene et al., 2011a). In addition, high frequency stimulation (125 Hz) but not low frequency stimulation (10 Hz) of the afferents causes activation of group I mGluRs in class 2 cells (Viaene et al., 2011a), consistent with the idea that mGluRs may be activated only under conditions of strong and repetitive sensory inputs.

Physiology of mGluRs in layer 4 of the AC

Whether group I mGluRs are involved in the excitatory responses of pyramidal cells in layer 4 depends on the source of the synaptic inputs. Although the thalamocortical input to layer 4 uses glutamate as the excitatory transmitter, there is no mGluR component in the EPSPs of layer 4 pyramidal neurons when the thalamocortical pathway is activated (Lee and Sherman, 2008). In contrast, activation of the intracortical pathway from layer 6 depolarizes layer 4 neurons via group I mGluRs (Lee and Sherman, 2009b). Interestingly, activation of postsynaptic group II mGluRs by glutamate release from the layer 6 input produces inhibition in layer 4 cells via activation of GIRK channels (Lee and Sherman, 2009a). Therefore, different mGluRs (group I versus II here) activated by the same pathway generates exactly the opposite effects on cellular excitability, providing bidirectional dynamic regulation and thus maintenance of homeostasis of cellular excitability. Similarly, at the synapses formed between the primary and the secondary AC, postsynaptic group I mGluRs facilitate while group II mGluRs inhibit cellular excitability of neurons. This effect occurs only for neurons that express small EPSPs, but not large EPSPs (Covic and Sherman, 2011), indicating synapse-specific activation of mGluRs. In addition, group II mGluRs strongly inhibit excitatory transmission of the thalamocortical pathway via presynaptic action (Lee and Sherman, 2012). Involvement of mGluRs in long-term plasticity in layer 4 neurons is also input pathway dependent. The non-specific mGluR antagonist MCPG did not affect long-term plasticity (LTP or LTD of field EPSPs) of excitatory transmission from layer 6 to layer 4 cells, suggesting the absence of mGluR involvement in long-term plasticity at this synapse (Watanabe et al., 2007). In contrast, group I mGluRs are required for the induction of LTD and LTP of EPSCs in cells recorded from layers 3 and 4 when the thalamocortical pathway is activated (Blundon et al., 2011; Chun et al., 2013).

Physiology of mGluRs in layers 5 and 6 of the AC

No mGluR component is found in the excitatory responses from the thalamic inputs to layers 5 and 6 (Viaene et al., 2011b).

The roles of mGluRs in the AC are complicated, but a common theme that seems to exist is that mGluR-mediated modulation depends on the combination of a number of factors including the sources of synaptic inputs, the cell types, and the location of the cells in different layers. Not surprisingly, mixed results were reported when cells were sampled from mixed layers. Future experiments need to take these issues into consideration in order to better understand the roles of mGluRs in AC circuits.

ANATOMY AND PHYSIOLOGY OF mGluRs IN AVIAN LOWER AUDITORY BRAINSTEM

The chicken auditory brainstem has been used for decades as an excellent model for studying mechanisms underlying auditory signal processing (reviewed in Rubel et al., 1990; Grothe, 2003; Burger et al., 2011). Because of the specialized anatomy and well-characterized functions, the auditory nuclei in this model system provide unique opportunity to study mGluR-mediated modulation in specific functional auditory circuits (Fig. 3). After entering the brainstem, the auditory nerve (8th n.) branches and innervates the cochlear nucleus angularis (NA) and nucleus magnocellularis (NM). Bilateral projections from the NM innervate the nucleus laminaris (NL). The NM and NL are involved in coding temporal information of sound, whereas the NA is primarily involved in coding sound intensity among other features. All three nuclei (NM, NL, and NA) receive feedback inhibition from the ipsilateral superior olivary nucleus (SON), which receives its excitatory inputs from the NL and NA. These auditory nuclei are structurally distinct and easily identified in brain slice preparations. Cells in the NM and NL are more homogenous compared to their mammalian counterparts, AVCN and medial superior olive (MSO), respectively. More importantly, the synaptic connections among these nuclei are largely intact in brain slice preparations. These features make the avian auditory brainstem an excellent model to study the physiology of mGluR-mediated modulation of neuronal properties.

Expression of mGluRs in the avian auditory brainstem

Anatomical expression of mGluRs in the avian auditory system has been reported as supportive data in a few physiological studies. Immunohistochemistry has revealed expression of group I mGluRs (both mGluR1 and mGluR5) in NM neurons (Zirpel et al., 2000). Group II mGluRs are expressed in NM (Tang et al., 2013), NL (Okuda et al., 2013), and NA neurons (Fig. 3), while no anatomical data are available for group III mGluRs. The methods for immunostaining of group II mGluRs presented here have been described in Tang et al. (2013). Briefly, we used a specific polyclonal antibody against both mGluR2 and mGluR3 (Abcam, Cambridge, MA). Vibratome-sliced free-floating sections (50 μ m in thickness) were incubated with the primary antibody (1:500) followed by incubation with the biotinylated anti-rabbit secondary antibody (1:400). Negative control experiments were performed with omission of the primary antibody.

Regulation of calcium homeostasis and protein synthesis by mGluRs in the NM

About ten years after mGluRs were discovered, Rubel and colleagues (Zirpel et al., 1994) reported that activation of mGluRs increases phosphatidylinositol metabolism in NM neurons. In the following years, mGluR-mediated regulation of Ca^{2+} signaling in NM

neurons was extensively studied, primarily by the same research group (Lachica et al., 1995; 1998; Kato et al., 1996; Zirpel and Rubel 1996; Zirpel et al., 1995, 1998; Kato and Rubel, 1999; Zirpel and Parks, 2001). The key conclusion from these studies is that mGluRs, especially group I mGluRs, play critical roles in regulating Ca^{2+} signaling and maintaining Ca^{2+} homeostasis and cell survival in NM neurons. Readers are referred to a comprehensive review covering these studies (Rubel and Fritzsche, 2002). Recently, we extended these studies by characterizing synaptic activity-induced Ca^{2+} signaling and confirming mGluR-induced increase of intracellular Ca^{2+} concentration in NM neurons (Wang et al., 2012).

Another parallel line of research by Hyson and colleagues has examined mGluR-mediated regulation of protein synthesis in NM neurons. Maintenance and survival of NM neurons depend on their afferent excitatory input from the auditory nerve (reviewed in Rubel et al., 1990). Deprivation of the auditory nerve input disrupts protein synthesis in NM neurons (Hyson and Rubel, 1989). This activity-dependent regulation of protein synthesis does not involve ionotropic glutamate receptors (Hyson, 1997), but rather relies on activity of mGluRs (Hyson, 1998). Specifically, group I and II mGluRs are required to maintain protein synthesis in NM neurons, because blocking either group I or II mGluRs eliminates activity-dependent regulation of ribosomes *in vitro* (Nicholas and Hyson, 2004) as well as *in vivo* (Carzoli and Hyson, 2011). These studies demonstrate the regulatory roles of mGluRs in Ca^{2+} signaling and protein synthesis, suggesting potential neuroprotective effects of mGluRs under abnormal hearing conditions.

Physiology of mGluRs in the NM

Previously a Ca^{2+} imaging study investigated postsynaptic mGluR-mediated modulation of Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCCs) in NM neurons (Lachica et al., 1995). It was important to confirm the observation with electrophysiological approaches that allow higher resolution in monitoring subtle changes in membrane Ca^{2+} currents. We thus characterized VGCC types using various blockers specific for different subtypes of VGCCs and showed that NM neurons possess both low- and high-threshold VGCCs, with N-type channels being dominant (Lu and Rubel, 2005). Activation of mGluRs with agonists that individually target each of the three groups of mGluRs reduces VGCC current (Lu and Rubel, 2005), indicating that multiple mGluRs are present on postsynaptic NM neurons and all of them are involved in regulating Ca^{2+} influx through membrane VGCCs.

Besides modulation of ion channels by postsynaptic mGluRs, regulation of synaptic transmission via presynaptic mGluRs has been commonly observed in the CNS (reviewed in Schoepp, 2001). A series of studies investigating mGluR-mediated modulation of synaptic transmission in the chicken auditory brainstem have been undertaken. The focus has been on mGluR-mediated heteroreceptor modulation of the inhibitory inputs to NM and NL. The inhibitory transmission in the avian auditory brainstem neurons bears an unusual feature in that it is depolarizing but potently inhibitory in NM (Hyson et al., 1995; Lu and Trussell, 2001; Monsivais and Rubel, 2001), NL (Tang et al., 2009), and NA (Kuo et al., 2009) neurons. The depolarized reversal potential of the inhibitory transmission in these neurons makes it possible that overdriving of the inhibitory afferent leads to generation of GABA-induced action potentials (Lu and Trussell, 2001; Kuo et al., 2009; Tang et al., 2009).

Because such spiking activity could disrupt phase-locking fidelity of the timing coding neurons in response to their excitatory inputs, multiple mechanisms have been proposed to prevent GABA-induced spiking. Regulation of the synaptic strength of the inhibition in NM neurons is achieved partially by intrinsic low-threshold K_v conductance (Monsivais and Rubel, 2001) and partially by a feedback mechanism via GABA_BRs (Lu et al., 2005). We have further shown that mGluRs contribute to the regulation of GABA release at NM through presynaptic mechanisms (Lu, 2007). Based on these results, we proposed a dual-modulation model stating that mGluRs exert a tonic modulation of GABA release in NM neurons, while GABA_BRs provide a feedback mechanism limiting over-activation of the inhibitory system.

One puzzling observation in the study by Lu (2007) was the incomplete recovery of IPSCs of NM neurons after being suppressed by activation of group II mGluRs with an agonist DCG-IV. At that time, we thought that the agonist might be non-reversible and bound with group II mGluRs permanently during the recording period. Further investigation through long (> 1 hr) recordings of IPSCs revealed that this is not the case. Rather, a novel form of long-term plasticity, group II mGluR-induced LTD at the GABAergic synapses, has been induced (Tang et al., 2013). Group II, but not group I and III mGluRs, induce the LTD of GABA release in NM neurons (Tang et al., 2013). Interestingly, mGluRs did not act as autoreceptors to modulate the excitatory transmission in NM (Otis and Trussell, 1996; Tang et al., 2013). However, whether there is mGluR-mediated modulation of EPSCs in low-frequency (LF) neurons needs to be further examined because these neurons are different from middle/high-frequency (MF/HF) neurons in terms of cellular morphology and physiology (Fukui et al., 2004, 2006; Oline and Burger, 2014), and LF neurons have been generally avoided in our previous recordings for consistency of sampled cell populations.

While *in vitro* studies have demonstrated short- and long-term mGluR-mediated modulation of ion channels and inhibitory transmission in NM neurons, *in vivo* studies examining roles of mGluRs in temporal coding in NM neurons are completely lacking. Based on our proposal that mGluRs control the inhibitory strength in NM neurons to prevent generation of GABA-induced spike that are not phase-locked to the excitatory input from the auditory nerve, we speculate that the phase-locking fidelity of NM neurons may be enhanced when mGluRs are activated. Conversely, blocking mGluRs may reduce phase-locking fidelity of NM neurons and consequently disrupt coincidence detection in NL neurons.

Physiology of mGluRs in the NL

Given the similarities in neuronal properties between NM and NL neurons, it is not surprising that the dual-modulation model proposed for NM also applies to NL neurons. Indeed, we confirmed this prediction by showing that both GABA_BRs and mGluRs modulate synaptic inhibition in NL neurons (Tang et al., 2009; Tang and Lu, 2012a). The difference in modulation between NM and NL is that group II and III mGluRs, but not group I mGluRs, are involved in the modulation at NL. Whether group II mGluRs induce LTD of GABA release in NL awaits further investigation. Although strong, the neuromodulation by mGluRs and GABA_BRs of the synaptic inhibition in NL neurons did not change the temporal profile of IPSPs evoked by electrical stimulation of the afferent inputs at high rates

(Lu, 2009). This is critical for the sustained GABAergic inhibition to function as a gain control mechanism in sharpening coincidence detection window in NL neurons. GABA_BRs reduce tonic inhibition via presynaptic modulation of GABA release (Tang et al., 2011), and mGluRs are expected to have similar effects because they, like GABA_BRs, reduce GABA release via presynaptic actions (Tang et al., 2009). Taken together, mGluR-mediated modulation of synaptic inhibition in NL is highly potent and dynamic, ensuring proper inhibitory strength in binaural hearing processing.

Modulation of the excitatory transmission by mGluRs in NL neurons awaits further clarification, because the reported results from different research groups are not completely in agreement. We reported that neither GABA_BRs nor mGluRs modulated the excitatory inputs in neurons sampled primarily from the MF and HF regions of NL in late embryos (Tang et al., 2009). Recently it was reported that mGluRs (mainly group II and possibly group III) suppressed EPSCs of NL neurons in chick hatchlings in a graded manner along the frequency axis of NL. Strong modulation occurred in LF neurons, less modulation was detected in MF neurons and even smaller modulation in HF neurons, and a graded expression of group II mGluRs along the frequency axis of NL supported the physiology (Okuda et al., 2013). The authors reasoned that the discrepancy between our observation and theirs is due to the use of animals of different age, and they showed that the expression of mGluRs in late embryos was weak. However, in late embryos mGluRs strongly modulate synaptic inhibition in NL (Tang et al., 2009; Tang and Lu, 2012a), and strong expression of group II mGluRs was detected in late embryos in NM (Tang et al., 2013) and NL (Fig. 4B; our unpublished data). Furthermore, we observed mGluR-mediated modulation of EPSCs in LF but not MF/HF NL neurons in both embryos and chicken hatchlings (our unpublished data). Therefore, it is not yet certain whether mGluRs modulate the excitatory transmission in NL neurons in a continuous graded manner as proposed by Okuda et al. (2013).

Synaptic excitation in NL neurons is tonotopically distributed in a graded manner (Fig. 4C), with EPSCs becoming faster and larger with increasing tuning frequency (Sanchez et al., 2010; Slee et al., 2010). In contrast, synaptic inhibition is not distributed in a graded manner (Fig. 4D), with fast IPSCs and minimal tonic inhibition in LF neurons but slow IPSCs and strong tonic inhibition in MF/HF neurons (Tang et al., 2011; Tang and Lu, 2012b; Yamada et al., 2013). Our working hypothesis is that neuromodulation is tonotopically distributed to improve synaptic integration at particular sound frequencies. Specifically, we propose that mGluR-mediated modulation of synaptic transmission in NL is tonotopically distributed in a manner complementary to the tonotopic distribution of synaptic excitation and inhibition (Fig. 4C, D). In other words, mGluRs modulate synaptic excitation in LF neurons, and the modulation rapidly diminishes in MF/HF neurons. In contrast mGluRs exert a graded modulation of synaptic inhibition, which has a less-graded distribution across the frequency axis. This complementary mode of neuromodulation could maintain a balance of excitation and inhibition in neurons across the frequency axis. Future studies should fully investigate this issue by sampling large cell populations from different frequency regions, and by using animals at various ages.

In vivo physiological experiments examining the roles of mGluRs in the NL are of great significance but currently completely lacking. The mechanisms underlying mGluR-mediated

modulation on coincidence detection and ITD coding in NL is likely to be highly dependent on frequency regions. In LF neurons, mGluRs may improve coincidence detection by sharpening the excitatory responses and regulating synaptic inhibition, whereas in MF/HF neurons, mGluRs may improve ITD coding primarily via controlling the inhibitory synaptic strength. It is plausible that mGluRs provide a gain control mechanism for ITD coding, resulting in neuronal adaptation in sound localization, similar to the modulatory function played by GABA_BRs in the MSO (Stange et al., 2013).

In contrast to the relatively extensively studied mGluR modulation in the avian timing coding pathway, the roles of mGluRs in the intensity coding pathway starting with the NA are unknown. The majority of NA neurons are believed to be multi-functional including encoding intensity of sounds (Köppl and Carr, 2003), and have large dynamic range (the range of sound intensity within which a neuron fires spikes between 5% and 95% of its maximal spike rate), suggesting of high sensitivity of NA neurons to changes in sound levels (Warchol and Dallos, 1990). Given that mGluRs are likely to be activated in response to repetitive stimuli at high intensity, it is likely that mGluRs play a role in shaping non-monotonic input-output functions in the NA. Blocking mGluRs may disrupt intensity coding in NA neurons and consequently reduce the precision for sound localization using interaural intensity difference as a cue in higher order auditory nuclei.

To summarize the results in the avian auditory system, postsynaptic mGluRs regulate Ca²⁺ signaling via modulating VGCCs and internal Ca²⁺ stores in NM neurons. Presynaptic mGluRs modulate synaptic transmission in NM and NL in a frequency region dependent manner. While modulation by mGluRs on synaptic inhibition is largely similar between NM and NL, modulation on synaptic excitation is more complicated. While mGluRs in the avian auditory system have been studied using *in vitro* methods, it is imperative to test how mGluRs affect auditory processing at the systems level using *in vivo* physiological methods. Taken together, as in the mammalian lower auditory brainstem, mGluRs in the avian auditory brainstem modulate neuronal properties through both pre- and postsynaptic mechanisms. However, a meaningful comparison of mGluR effects between the avian auditory brainstem neurons (in NM and NL) and their mammalian counterparts (AVCN bushy cells and MSO) cannot yet be formed, because there is little data on mGluRs for mammalian bushy cells in the AVCN, and literally there is no data on mGluRs for the MSO. This again points to the need for further in-depth investigation of mGluRs in the auditory system across different animal species.

CONCLUSIONS

One of the main goals of this review is to inspire interest and enthusiasm in studying anatomy and physiology of mGluRs in the auditory system. Many questions regarding the essential roles of mGluRs in auditory processing remain unanswered. Are mGluRs required for normal hearing? Do mGluRs play critical roles in the development of auditory circuits? How do mGluRs modulate auditory processing at the systems level? How are the structure and function of the auditory system affected if mGluRs are dysfunctional? Do the mechanisms underlying the protective effects of mGluRs found in other brain areas apply to the auditory system? If mGluRs provide protection against hearing loss caused by various

insults, what are the potentials of developing drugs targeting mGluRs to rescue and/or prevent hearing impairment? Each auditory station is composed of multiple cell types anatomically intertwined with each other. It remains a huge challenge to investigate the modulatory effects and mechanisms of mGluRs in particular cell types, especially at the systems and behavior levels. Strategies utilizing an array of approaches in multiple animal model systems are required in order to tackle these issues and shed light on the roles of mGluRs in auditory processing.

Key knowledge of the function of mGluRs in auditory processing could be gained from focusing on the following areas in future studies. First, in order to reveal the organization of mGluRs on the synaptic loci of different cell types, anatomical expression of mGluRs in the auditory system needs to be systematically investigated, at both the light and electron microscopy levels. Second, physiological studies on the functions of mGluRs in auditory processing should be expanded by using *in vivo* physiological recordings combined with pharmacological and morphological tools. It would be useful if the initial attempts focus on the auditory nuclei where extensive *in vitro* research has been done. Third, *in vitro* physiological studies in understudied auditory nuclei need to be performed to establish cellular mechanisms of mGluR neuromodulation. This will provide a foundational basis for investigating the functions of mGluRs at the systems and behavioral levels. Finally, pilot studies should be initiated to investigate potential roles of mGluRs in auditory disorders, such as hearing loss, tinnitus, and deafness.

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ABBREVIATIONS

AC	auditory cortex
AVCN	anteroventral cochlear nucleus
CN	cochlear nucleus
DCN	dorsal cochlear nucleus
EPSC/P	excitatory postsynaptic current/potential
GABA_BR	GABA _B receptor
GIRK	G-protein-coupled inward rectifier K ⁺
HF	high-frequency
IC	inferior colliculus
IHC	inner hair cell

IPSC/P	inhibitory postsynaptic current/potential
LF	low frequency
LSO	lateral superior olive
LTD/P	long-term depression/potential
MF	middle-frequency
MGB	medial geniculate body
mGluR	metabotropic glutamate receptor
MNTB	medial nucleus of trapezoid body
MSO	medial superior olive
NA	nucleus angularis
NL	nucleus laminaris
NM	nucleus magnocellularis
OHC	outer hair cell
PVCN	posteroventral cochlear nucleus
SON	superior olivary nucleus
VCN	ventral cochlear nucleus
VGCC	voltage-gated Ca ²⁺ channel

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Highlights

- Metabotropic glutamate receptors (mGluRs) are expressed in auditory structures.
- Intrinsic and synaptic properties of auditory neurons are modulated by mGluRs.
- Studies on mGluRs in hearing at the systems and behavior levels are warranted.

Fig. 1.

Schematic drawing summarizing the physiology of mGluRs in mammalian subcortical auditory structures. (A) Postsynaptic group I mGluRs participate in the excitatory transmission at the synapses between inner hair cells (IHC) and spiral ganglion (SG) neurons. Group II mGluRs may suppress GABA release of the lateral olivocochlear efferent system via a presynaptic mechanism. (B) Postsynaptic group II and III mGluRs contribute to the induction of long-term plasticity of the excitatory input in the dorsal cochlear nucleus (DCN). In the ventral cochlear nucleus (VCN), postsynaptic group I mGluRs increase the excitability of bushy cells. (C) In the medial nucleus of trapezoid body (MNTB), postsynaptic group I mGluRs mediate a retro-suppression of glutamatergic transmission, whereas presynaptic group III mGluRs inhibit glutamate release via modulating voltage-gated Ca^{2+} channels. The inhibitory input from the MNTB to the lateral superior olive (LSO) is modulated by presynaptic group II mGluRs, whereas the excitatory input from the cochlear nucleus (CN) to the LSO is modulated by presynaptic group II and III mGluRs. Postsynaptic group I and II mGluRs increase intracellular Ca^{2+} concentration in developing LSO neurons. (D) In the inferior colliculus (IC), presynaptic group II mGluRs suppress both the excitatory and inhibitory transmission. Postsynaptic group I mGluRs enhance while group II mGluRs suppress the cellular excitability. (E) Weak evidence suggests that postsynaptic group I and II mGluRs depolarize neurons of the medial geniculate body (MGB). It is worth noting that there are multiple cell types in most of the structures depicted here especially in the DCN, VCN, and the IC. The schematic drawing does not reflect this complexity, and limited information on the physiology of mGluRs in different cell types is available.

Fig. 2.

Schematic drawing showing mGluR-mediated modulation in the auditory cortex (AC). In pyramidal cells in layers 2/3, presynaptic mGluRs (possibly group I and II mGluRs) inhibit both excitatory and inhibitory inputs that originate from layer 6. Postsynaptic group I mGluRs are activated by the thalamocortical input and mediate membrane depolarization in class 2 cells (modulator neurons). In layer 4, group I mGluRs activated by the intracortical pathway from layer 6 depolarize pyramidal neurons, whereas postsynaptic group II mGluRs produce inhibition via activation of GIRK channels. Also in layer 4, presynaptic group II mGluRs produce strong inhibition of excitatory transmission of the thalamocortical pathway. In layers 3/4, group I mGluRs are required for the induction of LTD and LTP of the thalamocortical excitatory transmission. In this and subsequent figure, pre- and post-: presynaptic and postsynaptic, respectively. I, II, and III: group I, II, and III mGluRs.

Fig. 3. mGluR-mediated modulation in the avian auditory brainstem. (A) Coronal section of chicken brainstem showing immunohistochemical staining of group II mGluRs (our unpublished data). NA: cochlear nucleus angularis; NM: cochlear nucleus magnocellularis; NL: nucleus laminaris; SON: superior olivary nucleus. (B) Schematic drawing showing mGluR-mediated modulation in the avian auditory brainstem circuits. The auditory nerve (8th n.) bifurcates and innervates the NA and NM. The NL receives bilateral excitatory inputs from NM. All three nuclei (NM, NL, NA) receive feedback inhibition from the ipsilateral SON, which is driven by excitatory inputs from NL and NA. All three groups of mGluRs are involved in presynaptic modulation of the inhibitory transmission in NM neurons. Group II and III mGluRs suppress both excitatory and inhibitory transmission in NL, possibly in a tuning frequency-dependent manner. Group II and III mGluRs also modulate the excitatory transmission at NA (our unpublished data), without affecting the excitatory inputs to NM. Postsynaptic mGluRs modulate voltage-gated Ca²⁺ channels in NM neurons, whereas postsynaptic effects of mGluRs in NL and NA neurons have not been reported. Studies on mGluR-mediated modulation in the SON are completely lacking.

Fig. 4.

Tonotopically distributed neuronal properties in the nucleus laminaris (NL). (A) Schematic drawing showing that NL cells coding for higher sound frequencies have shorter and more dendrites. (B) There is a clear gradient in the protein expression of group II mGluRs, with strongest immunoreactivity in low-frequency (LF) neurons, and weaker immunoreactivity in middle-frequency (MF) and high-frequency (HF) regions (our unpublished data). Scale bar: 200 μ m. (C) Synaptic excitation is distributed in a graded manner, with excitatory postsynaptic currents (EPSCs) becoming faster and larger with increasing tuning frequency. (D) Synaptic inhibition is not distributed in a graded manner. LF neurons have fast inhibitory postsynaptic currents (IPSCs) and minimal tonic inhibition, whereas MF/HF neurons have slow IPSCs and strong tonic inhibition (MF and HF neurons do not differ). It is debatable whether mGluR-mediated modulation of synaptic transmission is graded for both excitation and inhibition. We propose that the mode of mGluR-mediated modulation is complementary to the distribution of synaptic excitation and inhibition. In other words, mGluRs modulate synaptic excitation in LF neurons, and the modulatory effect rapidly diminishes in MF/HF neurons. In contrast, mGluRs exert a graded modulation of synaptic inhibition, which has a less-graded distribution along the frequency axis. sIPSC: spontaneous IPSC.

Table 1
Summary of anatomical expression of mGluRs in the mammalian auditory system.

structure	mGluR Group			mGluR I		mGluR II			mGluR III			references
	I	II	III	1	5	2	3	4	6	7	8	
cochlea		n.d.		(+)	n.d.	n.d.	n.d.	n.d.	n.d.	++	n.d.	<i>1, 2</i>
CN												<i>3–13</i>
AVCN				++	n.d.	++	n.d.	(+)	n.d.	(+)	n.d.	
PVCN				++	n.d.	++	n.d.	(+)	n.d.	(+)	n.d.	
DCN				++	+/-	++	+	(+)	n.d.	(+)	n.d.	
SOC												
MNTB				++ [#]	n.d.	++ [§]	++ [§]	++ [‡]	n.d.	n.d.	n.d.	<i>14–16</i>
LSO	n.d.		n.d.	n.d.	n.d.	+	+	n.d.	n.d.	n.d.	n.d.	<i>17</i>
Others	n.d.	n.d.	n.d.									
IC		n.d.		(+)	(+)	n.d.	n.d.	+	(+)	+	+	<i>18, 19</i>
MGB			n.d.	++	n.d.	+	+	n.d.	n.d.	n.d.	n.d.	<i>20–22</i>
AC	n.d.		n.d.	n.d.	n.d.	+	+	n.d.	n.d.	n.d.	n.d.	<i>23, 24</i>

positive expression of mRNA, or protein, or both
n.d. no data
++ positive protein expression detected by immunohistochemistry or western blot
(+) positive mRNA expression detected by RT-PCR
+/- weak or no expression
+? unknown expression of specific mGluR member
[#] postsynaptic expression only
[§] both presynaptic and postsynaptic expression
[‡] presynaptic expression only

References:

1–2 Safieddine and Eybalin, 1995; Friedman et al., 2009.
3–13 Ohishi et al., 1995, 1998; Wright et al. 1996; Petralia et al., 1996a, 1997; Bilak and Morest, 1998; Bradley et al., 1998; Kinoshita et al., 1998; Kemmer and Vater, 2001; Irie et al., 2006; Dino and Mugnaini, 2008.

¹⁴⁻¹⁶ Elezgarai et al., 1999, 2001; Kushmerick et al., 2004.

¹⁷ Niedzielski et al. 2007.

¹⁸⁻¹⁹ Yip et al., 2001; Martinez-Galan et al., 2012.

²⁰⁻²² Martin et al., 1992; Fotuhi et al., 1993; Petralia et al., 1996b.

²³⁻²⁴ Lee and Sherman, 2009a, 2012.

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