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Chloride Homeostasis in Neurons With Special Emphasis on the Olivocerebellar System: Differential Roles for Transporters and Channels

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The intraneuronal ionic composition is an important determinant of brain functioning. There is growing evidence that aberrant homeostasis of the intracellular concentration of Cl^- ($[Cl^-]_i$) evokes, in addition to that of Na^+ and Ca^{2+}, robust impairments of neuronal excitability and neurotransmission and thereby neurological conditions. More specifically, understanding the mechanisms underlying regulation of $[Cl^-]_i$ is crucial for deciphering the variability in GABAergic and glycinergic signaling of neurons, in both health and disease. The homeostatic level of $[Cl^-]_i$ is determined by various regulatory mechanisms, including those mediated by plasma membrane Cl^- channels and transporters. This review focuses on the latest advances in identification, regulation and characterization of Cl^- channels and transporters that modulate neuronal excitability and cell volume. By putting special emphasis on neurons of the olivocerebellar system, we establish that Cl^- channels and transporters play an indispensable role in determining their $[Cl^-]_i$ and thereby their function in sensorimotor coordination.

Keywords: chloride homeostasis, chloride transporters and channels, GABAergic inhibition, olivocerebellar system, cerebellar motor learning

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CHLORIDE REGULATION IN BRAIN CELLS

Chloride (Cl^-) is the most abundant transportable anion in all cells of the body and it performs fundamental biological functions in all tissues. The intracellular concentration of chloride ($[Cl^-]_i$) is regulated and maintained by a delicate functional balance between the operations of plasma membrane Cl^- channels and those of transporters, as well as those of local impermeant anions (Rivera et al., 1999; Glykys et al., 2014). In the central nervous system, Cl^- channels and transporters play key roles in neuronal growth and development, neurotransmitter uptake, intracellular pH modulation, cell volume regulation and, perhaps most importantly, setting $[Cl^-]_i$ either above or below its equilibrium potential (Sangan et al., 2002; Deidda et al., 2014; Ruffin et al., 2014; Jentsch, 2016; Glykys et al., 2017). In addition, $[Cl^-]_i$ plays a crucial role in moderating neuronal excitability by determining the postsynaptic responses to the neurotransmitters GABA and glycine (Ben-Ari et al., 2007; Branchereau et al., 2016; Doyon et al., 2016). One of the most studied roles of $[Cl^-]_i$ in neurons is its modulatory function in postsynaptic responses evoked by activation of ligand-gated Cl^- channels, such as $GABA_A$ receptors ($GABA_ARs$) (Bormann et al., 1987). The direction of the Cl^- flow depends on the difference between the reversal potential of Cl^- (E_{Cl})

and the resting membrane potential (RMP) (**Figure 1B**). If E_{Cl} is negative compared to the RMP of the neuron, Cl^- flows inside the neuron. $GABA_A$Rs in these types of cells mediate inward (hyperpolarizing) Cl^- currents, which in turn lead to inhibition of the postsynaptic neuronal activity (**Figure 1Ba**). In contrast, if E_{Cl} becomes more positive compared to the RMP, outward (depolarizing) Cl^- flow through $GABA_A$Rs leads to excitation of the postsynaptic neuron (**Figure 1Bc**). Therefore, the activities of Cl^- channels and transporters that regulate $[Cl^-]_i$ are critical for determining the polarity of the impact of $GABA_A$Rs on the neuronal membrane potential.

Mutations or deletions of Cl^- channels and transporters in the brain have been linked to genetic disorders, such as particular forms of neonatal seizures and epilepsy, ataxia, hyperekplexia (startle disease), and autism spectrum disorders (Cohen et al., 2002; Vermeer et al., 2010; Pizzarelli and Cherubini, 2011; Deidda et al., 2014). In addition, impaired Cl^- homeostasis has been associated with pathology of the brain following acute injuries, such as hypoxic-ischemic encephalopathy, brain edema, and post-traumatic seizures (Galeffi et al., 2004; Jin et al., 2005; Pond et al., 2006; Papp et al., 2008). Therefore, targeting Cl^- channels/transporters has been investigated as a therapeutic tool for re-balancing neuronal $[Cl^-]_i$ and rescuing the consequential neurological symptoms. One example of such a Cl^- based intervention is dampening the elevation of $[Cl^-]_i$ following traumatic brain injury (TBI), so as to prevent further neuronal swelling, excitatory GABA signaling, and seizure susceptibility (Annegers et al., 1998; Hung and Chen, 2012). Developing drugs that specifically target Cl^- channels or transporters may thereby not only ameliorate the short-term pathological processes induced by TBI, but also the long-term behavioral consequences (Rungta et al., 2015; Ben-Ari, 2017).

Chloride channels and transporters may become activated in response to membrane potential changes (such as ClC-channels), intracellular Ca^{2+} signaling (such as anoctamin-channels), and changes in intracellular pH (SLC4 and SLC26). In addition, Cl^- is transported across the membrane by cation-chloride co-transporters (CCCs), like the Na^+-K^+-Cl^- cotransporter (NKCC1), and K^+-Cl^- cotransporters (KCCs). Investigating the impact of such a rich set of widely expressed ion channels/transporters on neuronal functioning is a complex matter, not in the least because of the heterogeneity of the neuronal populations and the diverse functional interactions of Cl^- channels/transporters with each other and other ion carriers.

To allow an in-depth review of the functionality of neuronal Cl^- channels and transporters, we focus here on their impact on the olivocerebellar system. This interconnected brain network has been investigated in detail over the past decades and the extensive anatomical, electrophysiological, and behavioral datasets provide a remarkably detailed view of the properties of olivocerebellar circuitry, rendering it a suitable model for studying the consequences of abnormalities in Cl^- homeostasis at the cellular and network level. In order to set the stage, we will first provide a synopsis on the anatomical blueprint of the olivocerebellar system and highlight several hotspots where Cl^- homeostasis has been shown to be crucial for proper functioning. Thereafter we will discuss in detail several families of Cl^- channels and transporters and provide a concise view of the *status quo* in experimental studies. Hereby we hope to guide future translational investigations that aim to improve therapeutic strategies of Cl^- based treatments.

SIGNIFICANCE OF CHLORIDE IN THE OLIVOCEREBELLAR NETWORK

The olivocerebellar system consists of three key regions: cerebellar cortex (CX), cerebellar nuclei (CN), and inferior olive (IO). A large part of the neuronal interactions in this network depends on GABAergic signaling (**Figure 1A**; Andersson et al., 1988; Angaut and Sotelo, 1989; De Zeeuw et al., 1989, 1998; Fredette and Mugnaini, 1991). The output of the cerebellar cortex is exclusively mediated by GABAergic Purkinje cells (PCs). Several of the PC's downstream target neurons in the CN are also GABAergic who in turn inhibit neurons in the IO and cerebellar cortex (Lefler et al., 2014; Ankri et al., 2015). Another source of inhibition in the cerebellar cortex is the molecular layer interneurons (MLIs), which are not only activated by synaptic excitation from granule cells, but also by non-synaptic glutamate-spillover from IO axons, i.e., climbing fibers (CFs). MLIs synapse on either the somatic or dendritic membrane of PCs and thereby control PC action potential firing patterns (**Figure 1A**; Szapiro and Barbour, 2007). Aberrant GABAergic signaling at any of these synapses has been shown to evoke abnormalities in acquisition, correction, and timing of movements and thereby disrupt motor behavior (Bengtsson and Hesslow, 2006; Wulff et al., 2009; Seja et al., 2012; Rahmati et al., 2016). To the same extent, impairments of PC activity have been recently linked to autistic traits and other non-motor behaviors (Tsai et al., 2012; Peter et al., 2016).

It is particularly well-documented that the MLI to PC input determines the regularity and frequency of PC action potential firing (Häusser and Clark, 1997; Wulff et al., 2009) and that the MLI-mediated inhibition depends on the $[Cl^-]_i$ of PCs (Seja et al., 2012; Rahmati et al., 2016). Therefore, malfunction or deletion of GABAergic inhibitory input from MLIs to PCs leads to altered temporal firing patterns of PCs and causes various behavioral phenotypes in animal models (Wisden et al., 2009; Wulff et al., 2009; Seja et al., 2012; Rahmati et al., 2016). Likewise, in the olivary neurons the $[Cl^-]_i$ modulates their excitability and thereby the excitation of PCs, CNs, and MLIs as mediated by their CFs (Szapiro and Barbour, 2007; Zhang et al., 2017). Altered neuronal excitability in IO evokes long-term changes in the activity of cerebellar neurons and the spatiotemporal firing pattern of the olivocerebellar network (De Zeeuw et al., 2011). The impact of $[Cl^-]_i$ on GABAergic signaling in the olivocerebellar circuitry is also remarkable for its role in controlling the electrical coupling among olivary neurons. It has been proposed that activation of the GABAergic input from the CN to the IO leads to a reduction of coupling, whereas blocking this input increases IO coupling (De Zeeuw et al., 2011; De Gruijl et al., 2014; Lefler et al., 2014). Thus, various cellular components of the olivocerebellar system appear highly sensitive to $[Cl^-]_i$ disruptions by mutations in plasma membrane Cl^-

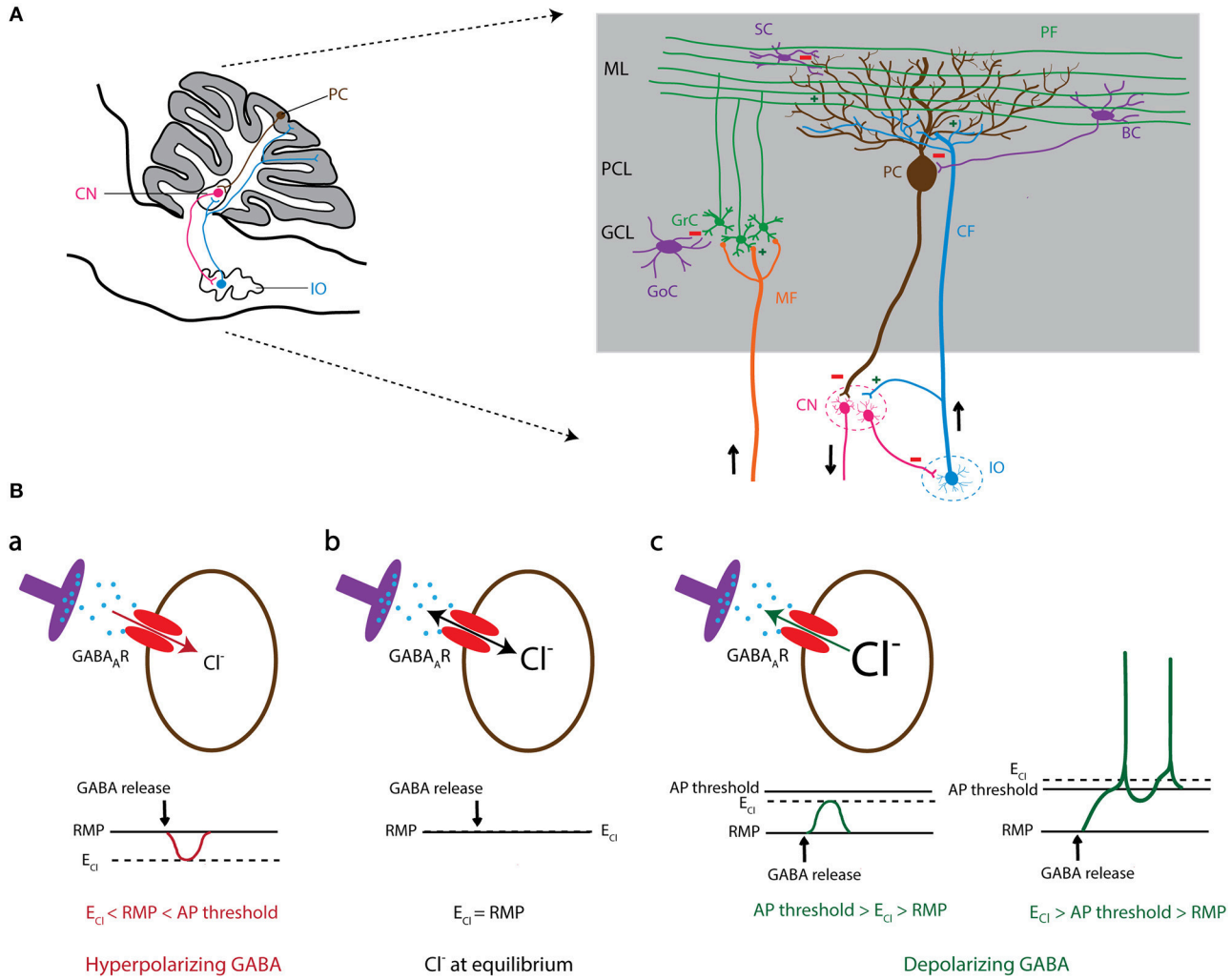

FIGURE 1 | GABAergic signaling in olivo-cerebellar circuit. **(A)** Schematic representation of a sagittal section of the mouse olivo-cerebellar system (left). Inferior olivary neurons (shown in blue) project to the cerebellar cortex (gray) and excite Purkinje cells (brown), as well as the deeply located cerebellar nuclei (CN) neurons (pink). A particular subset of CN neurons projects back to the inferior olive (IO), forming the olivo-cortico-nuclear loop. The right panel demonstrates the anatomical circuit of the cerebellar cortical neurons and their connectivity with CN and the IO. ML, molecular layer; PCL, Purkinje cell layer; GCL, granule cell layer; PC, Purkinje cell; GrC, granule cell; SC, stellate cell; BC, basket cell; GoC, Golgi cell; PF, parallel fiber; CF, climbing fiber; MF, mossy fiber; CN, cerebellar nuclei; IO, inferior olive. **(B)** The level of intracellular chloride concentration ($[Cl^-]_i$) dictates the polarity of the current through $GABA_A$ receptors ($GABA_A$Rs). If $[Cl^-]_i$ is low, the reversal potential of Cl^- (E_{Cl}) becomes negative compared to the resting membrane potential (RMP). In this condition $GABA_A$Rs mediate an inward Cl^- current that results in hyperpolarization of the cell membrane (a). In contrast, high $[Cl^-]_i$ results in a positive shift of E_{Cl} and leads to an outward Cl^- current through $GABA_A$R and depolarization of the cell membrane that potentially induces action potential firing (c). In conditions where E_{Cl} shifts to values similar to RMP, there will be no net Cl^- current through $GABA_A$Rs (b).

transporters and channels. Below we review the studies that investigated the effects of mutations and functional deletions of some of these proteins on $[Cl^-]_i$, which in many cases altered neuronal excitability, action potential firing patterns and motor coordination.

1. VOLTAGE-GATED CL$^-$ CHANNELS (CLC FAMILY)

ClC isoforms exhibit unique cellular expression patterns, with certain members (ClC-1 and ClC-2) primarily detected in plasma membrane, whereas some other members (ClC-3 to ClC-7) predominantly distributed in intracellular organelles and vesicles. Functional studies indicate that plasma membrane-bound ClCs operate in Cl^- channel mode and play a role in stabilizing membrane potential and/or Cl^- concentration across the membrane, while the intracellular organelles' ClCs function as electrogenic Cl^-/H^+ exchangers and facilitate endosomal and vesicular acidification (Jentsch et al., 2002; Jentsch, 2008, 2015; Bi et al., 2013). Many ClCs have not been studied in great detail for their function in the brain, but rather in other organs, including kidney where their malfunctions or deletions have been linked to various diseases in human (see **Table 1** for further references

TABLE 1 | ClC family of voltage-gated Cl^- channels.

ClCs	Expression	Function	Human disease	KO mouse	Pharmacology	References
ClC-1	Skeletal muscle, smooth muscle, heart, brain?	Stabilizing membrane potential in muscle	Myotonia congenita	Myotonia congenita, altered neuronal excitability	Inhibitors: Zn^{2+} and Cd^{2+}, 9-AC, DPC, and niflumic acid	Lorenzetto et al., 2009; Chen et al., 2013; Imbrici et al., 2015
ClC-2	Broad (brain, heart, muscle, kidney,...)	Transepithelial transport, cell volume control, neuronal excitability	Cardiovascular disease, epilepsy?	Leukoencephalopathy, degeneration of retina and testis, altered neuronal excitability	Inhibitors: Zn^{2+} and Cd^{2+}, DIDS, SITS	Smith et al., 1995; Clayton et al., 1998; Rinke et al., 2010; Ratté and Prescott, 2011
ClC-3	Broad (brain, heart, muscle, kidney,...)	Vesicular and endosomal acidification	?	Hippocampal neuronal degeneration, degeneration of retina		Kawasaki et al., 1994; Borsani et al., 1995
ClC-5	Kidney, intestine	Endosomal acidification	Dent's disease	Defects in renal endocytosis, proteinuria, hyperphosphaturia		Günther et al., 1998; Schwake et al., 2001; Vandewalle et al., 2001
ClC-6	Brain	Endosomal acidification	?	Lysosomal storage disease		Brandt and Jentsch, 1995; Poët et al., 2006
ClC-7	Broad (brain, bone, kidney,...)	Lysosomal Cl^- regulation, acidification of osteoclast, resorption lacunae	Osteopetrosis, neuronal ceroid lipofuscinosis, retinal degeneration, lysosomal storage disease	Lysosomal storage disease, retinal degeneration, osteopetrosis		Brandt and Jentsch, 1995; Kornak et al., 2001; Kasper et al., 2005
ClC-Kb	Kidney, inner ear	Transepithelial transport, salt reabsorption	Bartter syndrome type III	Salt loss, deafness	Inhibited by phenylbenzofuran carboxylic acids	Kobayashi et al., 2002; Jeck et al., 2004; Frey et al., 2006

and information regarding the members of the ClC-family). Below, we focus on the roles of ClC-1, ClC-2, and ClC-3 in the olivocerebellar system.

ClC-1

ClC-1, which is a plasma membrane-bound chloride channel encoded by the *CLCN-1* gene, is particularly known for its high Cl^- conductance, its expression in skeletal muscles, and its genetic mutations causing myotonia congenita (Jentsch, 2008). Recent studies have also identified mRNA and protein expression of ClC-1 in neuronal tissue, including pyramidal and dentate granule cells of the hippocampus, brain stem nuclei, thalamic nuclei, frontal neocortex, as well as cerebellar PCs (Chen et al., 2013; Imbrici et al., 2015). The presence of polymorphic alleles in *CLCN-1* gene in patients with idiopathic epilepsy underscores an important role for this Cl^- channel in neurological diseases (Chen et al., 2013). Although the precise pathophysiological mechanisms of ClC-1 channel mutations in epilepsy remain unknown, overexpression of ClC-1 in the inhibitory PCs has been found to hyperpolarize their resting membrane potential and reduce their excitability (Lorenzetto et al., 2009), which in turn may well lead to disinhibition of the CN and thereby influence epileptogenesis (Kros et al., 2015). Given that overexpression of ClC-1 even enhances inwardly rectifying Cl^- currents during depolarization in Xenopus oocytes, the impact on the membrane potential observed in PCs may also hold for other neurons (Steinmeyer and Klocke, 1991; Jentsch et al., 2002). One more unique impact of ClC-1 overexpression appears to be on synapse elimination. PCs overexpressing ClC-1 show a delayed elimination of their supernumerary CF inputs during development (Crepel et al., 1976; Hashimoto and Kano, 2005; Hashimoto et al., 2011); in normal wild type animals this process is finalized by the end of the third postnatal week, whereas in ClC-overexpressing transgenic mice it lasts at least 3 months (Lorenzetto et al., 2009). Thus, these studies provide supportive evidence for a contribution of voltage-gated Cl^- channels to the maturation of neuronal networks and neuronal excitability, and suggest that their function is critical to prevent neurological disorders such as epilepsy (Imbrici et al., 2015).

ClC-2

ClC-2, which is a plasma membrane-bound chloride channel encoded by the *CLCN-2* gene, is broadly expressed in the body with a wide range of functions, including regulation of cell volume and extracellular pH. In the brain, ClC-2 is expressed in different types of neurons, including pyramidal cells of the hippocampus and PCs of the cerebellum (Smith et al., 1995; Clayton et al., 1998), as well as in glia cells, like Bergmann glia in the cerebellar cortex (Sík et al., 2000; Blanz et al., 2007; Planells-Cases and Jentsch, 2009). In olivocerebellar system, ClC-2 knockout mice show the typical progressive spongiform vacuolation of their white matter tracts, which in the rest of the brain is manifested as leuko-encephalopathy (Blanz et al., 2007). Although no study has yet specifically examined the cell physiological role of ClC-2 in cerebellar neurons, several studies have evaluated its function in mediating the inwardly rectifying Cl^- current in hippocampal pyramidal cells (Weinreich and Jentsch, 2001; Rinke et al., 2010). Under conditions of high

$[Cl^-]_i$, i.e., those found in dorsal root ganglion cells and hippocampal neurons of rats with temporal lobe epilepsy, ClC-2 channels have been shown to extrude Cl^- (Staley et al., 1996; Ge et al., 2011). Endogenously, neuronal ClC-2 is open at resting membrane potentials and it does not inactivate or close at a given time upon activation (Staley et al., 1996). Thereby, it also has a profound effect on the membrane resistance, action potential threshold, and neuronal excitability (Madison et al., 1986; Rinke et al., 2010; Ratté and Prescott, 2011). However, ClC-2 knockout mice do not show lowered seizure susceptibility levels in their temporal lobe compared to their wild type littermates (Rinke et al., 2010). One possible explanation may be that the hyperexcitability of part of their neurons was balanced out by increased excitability of their local inhibitory interneurons (Rinke et al., 2010). Similar compensatory mechanisms may also occur in the olivocerebellar network, as ClC-2 KO mice do not show obvious abnormalities in movement performance (Blanz et al., 2007). Alternatively, it is also plausible that up and/or downregulation of other Cl^- channels (for instance ClC-1) can compensate for deletion of ClC-2.

ClC-3

ClC-3, which is a Cl^-/H^+ exchanger encoded by the *CLCN-3* gene, is broadly expressed in many tissues, including brain, kidney, skeletal muscles, heart, and liver (Kawasaki et al., 1994; Borsani et al., 1995; Jentsch et al., 2002). It shows ubiquitous expression throughout the brain with some of the highest levels of expression in hippocampus and cerebellum (2004 Allen Institute for Brain Science; Allen Human Brain Atlas. Available from: mouse.brain-map.org). With regard to the cellular distribution of ClC-3, the existing literature points to predominant expression in endosomal compartments and synaptic vesicles where it contributes to acidification by mediating the exchange of Cl^- against protons (Stobrawa et al., 2001). However, its expression in plasma membrane has been the subject of conflicting reports (Li et al., 2000; Jentsch et al., 2002). Recent studies by Nelson and colleagues showed that ClC-3 is expressed in the plasma membrane of postsynaptic hippocampal neurons, where it is functionally linked to NMDA receptors and activated by CaMKII (Wang et al., 2006; Farmer et al., 2013). In addition, ClC-3 may participate in controlling Ca^{2+} influx and plasticity in hippocampal neurons (Farmer et al., 2013). ClC-3 knock-out mice show postnatal degeneration of the retina and hippocampus (Stobrawa et al., 2001). In the cerebellum, expression is high in PCs (2004 Allen Institute for Brain Science; Allen Human Brain Atlas. Available from: mouse.brain-map.org). According to the studies by Farmer and colleagues, it is tempting to hypothesize that ClC-3 may also play a role in controlling plasticity at the PF to PC synapse by reducing Ca^{2+} influx. However, it has not been established yet whether ClC-3 is also expressed in the plasma membrane of PCs.

2. CA^{2+}-ACTIVATED CL^- CHANNELS (ANOCTAMINS)

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) plays a vital role in cellular signal transduction pathways, neurotransmitter release, as well as cellular excitability (Fakler and Adelman, 2008; Greer and Greenberg, 2008). Voltage-gated Ca^{2+} channels, which open upon membrane depolarization, form the main source of Ca^{2+} influx, but intracellular Ca^{2+}-stores also contribute to elevating $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$ is the prime activator for Ca^{2+}-activated ion channels, including the large- (K_{Ca}, BK) and small-conductance (K_{Ca}, SK) Ca^{2+}-activated K^+ channels. BK and SK channels are well-known for their predominant effects on the repolarization of the membrane following an action potential, influencing intrinsic excitability and shaping the postsynaptic currents, such as those of dendritic Ca^{2+} spikes (Fakler and Adelman, 2008). In addition to the K_{Ca} channels, several *in vitro* electrophysiological studies have shown the existence of a range of Ca^{2+}-activated Cl^- currents (Cl_{Ca}), which can, depending on the $[Cl^-]_i$, depolarize or repolarize the membrane potential. One of the main families of Ca^{2+}-activated Cl^- channels is the TMEM16 family (also referred to as anoctamins), which contains 10 members (Ano1-10), most of which are present in many different cell types in the body. In addition to their ion channel activity, anoctamins have been implicated in a wide range of physiological tasks, such as phospholipid scrambling or regulation of specific K^+ channels (Suzuki et al., 2010; Huang et al., 2013a,b; Picollo et al., 2015). As a consequence, anoctamins have been attributed to various functionalities, such as smooth muscle contraction, olfactory and sensory signal transduction, and neuronal excitability (Picollo et al., 2015). Not surprisingly, the clinical implications of impaired TMEM16 activity is equally diverse in that patients with mutated anoctamins are associated with cancer (Duvvuri et al., 2012; Liu et al., 2012; Ubby et al., 2013; Guan et al., 2016), muscular dystrophy (Griffin et al., 2016), Scott syndrome (Suzuki et al., 2010), and autosomal recessive cerebellar ataxia (Vermeer et al., 2010).

In line with their de- or repolarizing impact on the membrane potential, opening of anoctamins leads to Cl^- efflux or influx depending on the gradient of Cl^- across the cell membrane. For instance, in olfactory and dorsal root ganglion (DRG) neurons with high $[Cl^-]_i$, Cl^- efflux through anoctamins amplifies the sensory signal transduction by depolarizing the cell (Stephan et al., 2009; Cho et al., 2012). In contrast, in hippocampal pyramidal cells and IO neurons, which typically have relatively low $[Cl^-]_i$, anoctamins cause hyperpolarization by mediating Cl^- influx (Huang et al., 2012; Zhang et al., 2017). It should be noted that not all anoctamins have yet been characterized as a Cl_{Ca} channel. So far Ano1, Ano2, and Ano6 have been shown to gate Cl^- dependent on Ca^{2+}-activation, albeit with variable affinities for Ca^{2+}. For example, Ano1 exhibits a higher affinity for Ca^{2+} and a longer de-activation time than Ano2, while Ano6 appears to have a very low affinity for Ca^{2+} (Pifferi et al., 2009; Stephan et al., 2009; Grubb et al., 2013).

Anoctamins start gating Cl^- fluxes upon a rise in $[Ca^{2+}]_i$ evoked by the opening of voltage-gated Ca^{2+} channels and release from internal Ca^{2+}-stores. Both Ano1 and Ano2 have been shown to interact with Ca^{2+} driven calmodulin complexes (Verkman and Galietta, 2009; Jung et al., 2013; Vocke et al., 2013). Additional layers of complexity are added by the observations that Ano1 does not have to be activated by a postsynaptic Ca^{2+} influx *per se*, but can also be activated locally

TABLE 2 | TMEM16 (Anoctamin) family.

Anoctamins	Expression	Function	Human disease	KO mouse	Pharmacology	References
TMEM16A (Ano1)	Epithelial tissue, smooth muscle, interstitial cells of Cajal, dorsal root ganglion neurons	Cl_{Ca} channel, involved in fluid secretion, muscle contraction, gastrointestinal contractility, pain processing	Tumor growth, cystic fibrosis, asthma	Low blood pressure	Inhibitors: CaCCinh-A01, CaCCinh-B01, Niflumic acid (NFA), and NPPB, agonists: INS37217	Chen et al., 2007; Yang et al., 2008; Huang et al., 2009; Stöhr et al., 2009; Namkung et al., 2011; Jin et al., 2013; Neureither et al., 2017
TMEM16B (Ano2)	Brain (hippocampal and thalamocortical neurons, olfactory bulb, inferior olive, Purkinje cells), retina, muscle	Cl_{Ca} channel, involved in neuronal excitability, olfactory and sensory signal transduction and smooth muscle contraction	?	Impaired motor behavior, partial reduction of electrical response to odorants, normal olfaction	Inhibitors: CaCCinh-A01, CaCCinh-B01, Niflumic acid (NFA) agonists: INS37217	Stöhr et al., 2009; Billig et al., 2011; Huang et al., 2012; Dauner et al., 2013; Zhang et al., 2015, 2017; Ha et al., 2016
TMEM16C (Ano3)	Brain (dorsal root ganglion cells), blood vessels, lung	Phospholipid scrambling, K_{Na} channel regulator	Craniocervical dystonia, tremor, asthma	Impaired endoplasmic reticulum-dependent Ca^{2+} signaling		Charlesworth et al., 2012; Huang et al., 2013a; Suzuki et al., 2013; Miltgen et al., 2016
TMEM16E (Ano5)	Muscle, bone, sperm	Phospholipid scrambling	Muscular dystrophy, gnathodiaphyseal dysplasia			Katoh, 2004; Tsutsumi et al., 2004; Gyobu et al., 2016
TMEM16F (Ano6)	Blood vessels, endosomes, brain?	Phospholipid scrambling, blood coagulation, $SCAN_{Ca}$ channel, Cl_{Ca} channel, involved in membrane excitability	Scott syndrome	Lysosomal storage disease		Suzuki et al., 2010, 2013; Yang et al., 2012; Grubb et al., 2013; Shimizu et al., 2013; Yu et al., 2015
TMEM16J (Ano9)	Epithelial cells, colonic tissue	Phospholipid scrambling	Colorectal carcinoma			Suzuki et al., 2013; Li et al., 2015
TMEM16K (Ano10)	Epithelial cells, cerebellum?	Intracellular protein involved in intracellular Ca^{2+} signaling, essential for apoptosis	Autosomal recessive cerebellar ataxia			Vermeer et al., 2010; Renaud et al., 2014; Chamard et al., 2016; Mišković et al., 2016; Wanitchakool et al., 2017

by interacting with Ca^{2+} dependent G protein-coupled receptor signaling and compartmentalized Ca^{2+} (Jin et al., 2013; Courjaret and Machaca, 2014). Not surprisingly, also this affinity for the modulation by compartmentalized Ca^{2+} signals from internal stores is likely differentially modulated between anoctamin family members. The diverse functionalities, together with the differentially distributed expression among cell types (**Table 2**), reveal a picture where it seems likely that anoctamin members are expressed as a function of cell-specific Ca^{2+} dynamics.

Neurons are characterized by continuously changing local $[Ca^{2+}]_i$. Therefore, it is important to understand how these changes in intracellular milieu influence the responses of anoctamins. In hippocampal neurons, TMEM16B (Ano2) has been shown to affect action potential generation through a Ca^{2+} induced suppression of excitatory postsynaptic potentials in dendrites, which in turn lowers probability of action potential generation (Huang et al., 2012). In support of these data, a recent study performed in thalamic neurons demonstrated that Ano2 contributes to spike-frequency adaptation in thalamic neurons (Ha et al., 2016). In this study, knockdown of Ano2 reduces inter-spike interval lengths, resulting in a higher firing rate. The authors conclude that in the thalamus the main function of Ano2 is to drive hyperpolarizing currents as a consequence of depolarization induced $[Ca^{2+}]_i$ increase. The difference between SK K_{Ca} and Ano2 Cl_{Ca} mediated hyperpolarization in thalamic cells was hypothesized to be due to different decay time kinetics between these channels, in that Ano2 has a longer decay time duration and thus stronger influence on spike-frequency adaptation. In addition, a behavioral relevance for the Ano2 mediated spike-frequency adaptation was revealed in thalamic neurons, as the mice that have Ano2 knockdown in the thalamus experience an increase in pain responses. From these data, the authors proposed that the Ano2-driven modulation of spike frequency adaptation may provide thalamic cells with the ability to suppress excessive thalamo-cortical transmission, which tunes the network sensitivity to sensory inputs that reach the thalamic complex.

In contrast to the previously discussed increase of excitability in thalamic neurons, a recent study on the role of Ano2 in IO cells reported that functional deletion of Ano2-channels resulted in decreased excitability of IO neurons (Zhang et al., 2017). In light of the typical IO activity pattern, which is dominated by oscillatory fluctuations of membrane potentials partially driven by Ca^{2+}-currents, the impact of Ano2-mutations

can be substantial. The so-called high threshold spikes are formed when high threshold Ca^{2+} channels are activated that allow for a large after-depolarization potential (ADP) upon which additional spikes (spikelets) can be detected (Llinás and Yarom, 1981a,b). In a study by Zhang et al. (2017) this ADP was shown to be prolonged in mouse mutants lacking Ano2. In addition, the IO cells showed prolonged AHP duration, which coincided with less spiking upon current injection. They hypothesized that the loss of the hyperpolarizing Cl_{Ca} current in the Ano2 deficient mice leads to prolonged activation of K_{Ca} SK channels and therefore to prolonged AHP duration, which in turn reduces the likelihood for action potential firing. The authors conclude that the role for Ano2 in IO cells seems to primarily function as a repolarizing current of the voltage gated Ca^{2+} currents and as such determines the ADP length to a rather large degree.

Other mechanisms by which anoctamins could potentially regulate neuronal excitability in the cerebellar system has been touched upon by recent studies in PCs, where Cl_{Ca} channels were found to be involved in depolarization-induced depression of inhibition (Satoh et al., 2013; Zhang et al., 2015). Here, the authors reported Ano2-induced reduction of GABAergic transmission through an increase of postsynaptic $[Cl^-]_i$ that reduces the driving force for Cl^- influx. As the IO is an integrated part of the olivo-cerebellar circuit, which has been shown to be critical for motor coordination and learning, Zhang et al. (2017) hypothesized that a less excitable IO due to the absence of Ano2 would lead to less input to PCs and as a consequence impaired motor learning. Zhang et al. (2017) investigated the behavioral consequences of Ano2 absence and found that Ano2 knockout mice had significant deficits in their motor learning performance during classical eyeblink conditioning, a cerebellar dependent task where a conditioned stimulus (light/sound) predicts the arrival of an unconditioned stimulus (air puff). However, the idea that a cerebellar related dysfunction in behavior can solely be due to IO expression of Ano2 has recently been put to question (Neureither et al., 2017). In this study, Neureither et al. (2017) proposed the previously mentioned depolarization-induced depression of inhibition in PCs to be the main cause for the motor deficits found in their *Ano2* knockout model. In this respect, it is important to emphasize that even though the Ano2 protein is abundantly expressed in the IO, there is also evidence for its expression in other brain areas involved in motor function, including the thalamus (**Table 2**). Further research will have to be conducted to determine whether the dysfunctional cerebellar related motor behaviors described in these studies can be reproduced in the conditional removal of Ano2 in the IO and/or PCs.

3. PH-SENSITIVE CL^- CHANNELS AND TRANSPORTERS

Acid-base regulation is a homeostatic mechanism, which is crucial for cell survival and function in all tissues. All vertebrates generate significant amount of acid via metabolism. To buffer the metabolic acid load, increasing the concentration of systemic bicarbonate (HCO_3^-) aids the cell's capacity to extrude acid (H^+). The transmembrane transport mechanisms for pH regulation include Cl^-/HCO_3^- exchangers, Na^+/H^+ exchangers, and Na^+/HCO_3^- cotransporters. These transporters are expressed in all cell types, including neurons and glia and are localized at the plasma membrane and membranes of the intracellular organelles (Alka and Casey, 2014). Studies on hippocampal neurons have reported an association between intracellular pH changes with neuronal excitability, in a way that a rise in the intracellular pH leads to increased neuronal excitability, while a fall in pH has the opposite effect (Balestrino and Somjen, 1988; Tombaugh and Somjen, 1996). It has been suggested that pH-induced neuronal activity may be related to the activation of NMDA receptors, which are highly pH sensitive and show increased open probability at alkaline pH (Tang et al., 1990; Traynelis and Cull-Candy, 1990; Majumdar and Bevensee, 2010). Here, we emphasize the expression, localization, and functional significance of Cl^-/HCO_3^- exchangers of the SLC4 and SLC26 families, which are involved in regulation of both intracellular pH and $[Cl^-]_i$.

3.1. SLC4 Family

One of the well-known families of HCO_3^- transporters is the SLC4 family of Cl^-/HCO_3^- exchangers, which is widely expressed in the body. This family contains 10 members (SLC4A1-5 and A7-11), with some mediating Na^+-independent Cl^-/HCO_3^- exchange (Anion Exchanger 1-3 or AE1-AE3) and some isoforms facilitating Na^+-dependent Cl^-/HCO_3^- exchange (NCBE and NDCBE). AE transporters mediate HCO_3^- extrusion while transporting Cl^- inside the cell. In contrast, NCBE and NDCBE transport HCO_3^- inside and Cl^- outside of the cell (**Figure 2**). The Cl^-/HCO_3^- exchangers have been shown to be important for baseline intracellular pH regulation, as well as facilitation of recovery after pH modifications (Hentschke et al., 2006; Jacobs et al., 2008). Three members of the SLC4 family are expressed in the olivocerebellar system, including SLC4A3 (AE3), SLC4A8 (NDCBE), and SLC4A10 (NCBE) (Chen et al., 2008; Burette et al., 2012). Here we review the literature on AE3 and NCBE, which have been studied for their functional roles.

SLC4A3 (AE3)

AE3 is an anion exchanger expressed in a wide variety of cells, which include for example particular types of excitable cells in the retina, heart, and brain (Alka and Casey, 2014). Similar to NKCC1, AE3 is considered as one of the main Cl^- accumulators in neurons by mediating the electroneutral exchange of one Cl^- while extruding one HCO_3^- (**Figure 2**). In mammals, there are two variants of the *SLC4A3* gene product: bAE3, which is abundant in the brain and retina, and cAE3, which is highly expressed in the cardiac tissue (Hentschke et al., 2006; Romero et al., 2013). bAE3 protein has been found in the hippocampus, cerebral cortex, cerebellum, and brainstem (Romero et al., 2013). In hippocampal pyramidal cells elevated pH levels activate bAE3, which leads to HCO_3^- extrusion, a function essential for recovery of intracellular alkalosis (Hentschke et al., 2006). There are reports on single nucleotide polymorphisms that occur in SLC4A3 gene and are predicted to impact the protein sequence of bAE3 at the extracellular loop and that may promote a higher

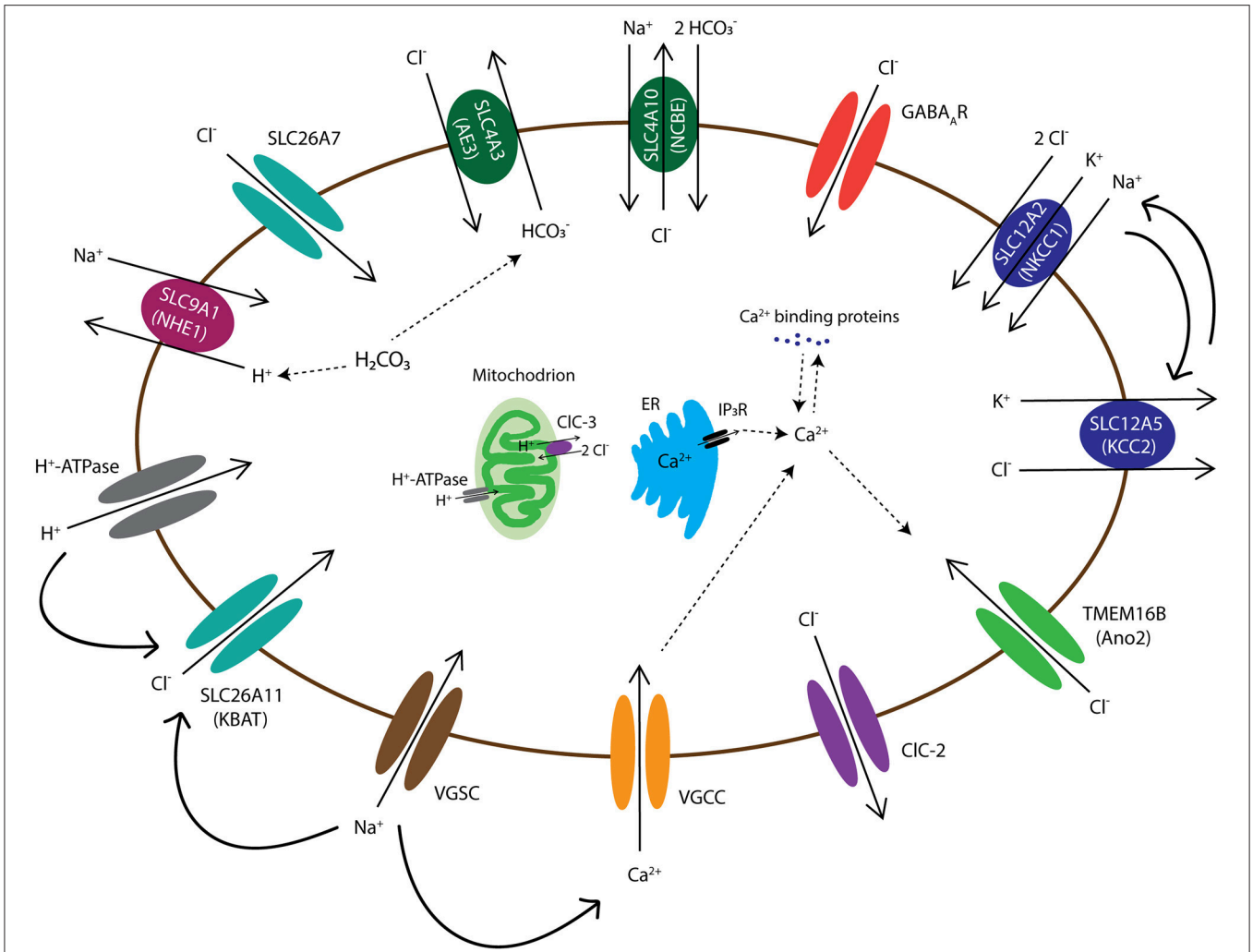

FIGURE 2 | Diagram of chloride channels and transporters that are expressed in the olivocerebellar neurons. The intracellular Cl^- concentration in all neurons, including the neurons of cerebellum and inferior olive, is maintained by activation of various transmembrane anion channels and transporters. These anion transporters/channels also interact with H^+ exchangers/channels, such as NHE1 and H^+-ATPase, and thereby are involved in intracellular pH regulation. Cell membrane depolarization, through voltage-gated Na^+ channels (VGSCs), activates several Cl^- channels/transporters, such as SLC26A11 (KBAT), to repolarize the cell by mediating inward Cl^- currents. In addition, depolarizations activate voltage-gated Ca^{2+} channels (VGCCs) and the resulting rise in intracellular Ca^{2+} levels, which may be aided by internal Ca^{2+} stores, may lead to activation of Ca^{2+} sensitive Cl^- channels, such as anoctamin-2 (Ano2). Transmembrane movements of Cl^- together with cations such as K^+ and Na^+ also control cell volume through transport of water molecules. Channels and transporters which belong to the same family of proteins are shown with a similar color.

sensitivity to idiopathic generalized epilepsy (Sander et al., 2002). While AE3 knockout mice appeared to be normal, they were affected by a lower seizure threshold and higher mortality rates after exposure to bicuculline, pentylenetetrazole, or pilocarpine (Hentschke et al., 2006). It is hypothesized that the increased seizure susceptibility of AE3 knockout mice is due to increased $[HCO_3^-]_i$ in hippocampal pyramidal neurons (Hentschke et al., 2006). Given that $GABA_A$Rs are also permeable to HCO_3^-, it may be that the observed phenotype of AE3 knockout mice, i.e., reduced GABAergic inhibition, is due to an increased $[HCO_3^-]_i$ (Hentschke et al., 2006). However, a lack of AE3 exchanger should also result in decreased $[Cl^-]_i$ and enhanced GABAergic inhibition. To identify the cellular mechanisms underlying the involvement of AE3 in neuronal excitability in future studies, one may have to combine Cl^- and pH measurements in neurons lacking AE3, as well as perform RNA-seq and DNA microarray to study the possibility of genetic compensations.

SLC4A10 (NCBE)

NCBE functions as a Na^+-dependent Cl^-/HCO_3^- exchanger. It is expressed in the olfactory bulb, cerebral cortex, brain stem, spinal cord, and cerebellum (Jacobs et al., 2008). In the cerebellum, it is densely expressed in PCs (Liu et al., 2011; Romero et al., 2013). Similar to SLC4A8 (NDCBE), NCBE may mediate the inward transport of Na^+ and HCO_3^- in exchange for intracellular Cl^- (**Figure 2**). Various studies have proposed that modest

levels of intracellular acidification can lead to termination of seizure-related activities (Chesler and Kaila, 1992; Zhan et al., 1998; Tong and Chesler, 1999; Jacobs et al., 2008). Using a global NCBE knockout mouse, Jacobs and colleagues found a significant increase in seizure threshold, supporting the impact of reduced HCO_3^- uptake and prolonged intracellular acidosis on seizure generating processes (Jacobs et al., 2008). NCBE knockout mice showed normal locomotor activity, as well as motor and spatial learning (Jacobs et al., 2008). MRI analysis of knockout mice indicated a significant reduction in the volume of brain ventricles compared to littermate controls. One of the reasons for collapsed ventricles could be an increase in intracranial pressure due to water accumulation in the brain parenchyma (Jacobs et al., 2008). However, NCBE knockout mice did not show any other anatomical signs of increased intracranial pressure (Jacobs et al., 2008). Jacob and colleagues concluded that NCBE can be considered as a new target for treatment of epilepsy.

How the $[Cl^-]_i$ and the pH regulation by Cl^-/HCO_3^- transporters interact in the olivocerebellar system remains to be investigated. However, studies on other channels and transporters sensitive to pH levels (e.g., Acid-Sensing Ion Channels or ASICs) have indicated a modulatory role for pH in neurotransmission and neuronal plasticity via influencing the activity of ionotropic and metabotropic glutamate receptors in both cerebellar and extracerebellar neurons (Allen and Attwell, 2002; Jovov et al., 2003). Tissue distributions and functions of SLC4 isoforms with Cl^-/HCO_3^- activity are summarized in **Table 3**.

3.2. SLC26 Family

The SLC26 family of anion exchangers consists of 10 members (SLC26A1-A11). Each SLC26 isoform has different modes of ion transport activities, including the exchange of Cl^- for various other molecules (bicarbonate, hydroxyl, sulfate, formate, iodide, or oxalate) and the formation of Cl^- channels (Rahmati et al., 2013; Soleimani, 2013). Mutations in human SLC26 genes can cause several autosomal recessive diseases, such as chondrodysplasias (by mutations in A2), chloride diarrhea (A3), and deafness and enlargement of the vestibular aqueduct in the Pendred syndrome (A4) (Hästbacka et al., 1994; Everett et al., 1997). Several mouse models of SLC26-family members have confirmed the wide range of tissue specific deficits (**Table 4**). Although studies have demonstrated the important roles of SLC26 family in different tissues, there are only few reports describing their expression patterns and functions in the brain. Here, we review the most recent findings for SLC26A7 and SLC26A11, which have been studied by utilizing knock-out mouse models.

SLC26A7

SLC26A7 functions as a Cl^- channel, which is regulated by intracellular pH (Kim et al., 2005). It can also operate as Cl^-/HCO_3^- exchanger, which plays a role in cell volume regulation during hypertonicity (Petrovic et al., 2003, 2004; Soleimani, 2013). SLC26A7-null mice show deficits in acid secretion in both kidney and stomach (Soleimani, 2013). In the brain, SLC26A7 is expressed in several regions including hippocampus and cerebellum, with the highest expression level in cerebellar PCs (Rahmati, 2015). In PCs SLC26A7 is densely expressed in both soma and dendrites. At the subcellular level, it is expressed both in cell membrane and intracellular compartments, but the function of SLC26A7 in cerebellar neurons remains to be elucidated. The locomotor activity of global SLC26A7 knockout mice is altered (smaller step size) compared to their wild type controls (Rahmati, 2015). One potential cause for this behavioral abnormality could be that the lack of SLC26A7 disrupts $[Cl^-]_i$ and/or intracellular pH, which in principle could disrupt cerebellar activity patterns. Future experiments should address this hypothetical cascade.

SLC26A11 (KBAT)

SLC26A11, also referred to as the "kidney brain anion transporter" (KBAT) due to its high expression levels in the kidney and brain, has been identified to operate as a Cl^-/HCO_3^- exchanger, Cl^-/SO_4^{2-} exchanger, or Cl^- channel (Xu et al., 2011; Rahmati et al., 2013; Soleimani, 2013). KBAT is expressed in

TABLE 3 | SLC4 family of anion transporters.

SLC4	Expression	Function	Human disease	KO mouse	Pharmacology	References
SLC4A1 (AE1)	Erythrocytes, kidney, heart, colon	Cl^-/HCO_3^- exchanger	Hemolytic anemia, distal renal tubular acidosis		Inhibitor: DIDS	Bruce et al., 1997; Jarolim et al., 1998; Karet et al., 1998; Shayakul and Alper, 2004; Stehberger et al., 2007; Romero et al., 2013
SLC4A2 (AE2)	Most epithelial cells	Cl^-/HCO_3^- exchanger	Osteopetrosis		Inhibitor: DIDS	Gawenis et al., 2004; Romero et al., 2013
SLC4A3 (AE3)	Brain, kidney, GI tract, smooth muscle and heart	Cl^-/HCO_3^- exchanger	Epilepsy, blindness	Lower seizure threshold	Inhibitor: DIDS	Sander et al., 2002; Hentschke et al., 2006; Romero et al., 2013; Ruffin et al., 2014
SLC4A8 (NDCBE)	Brain, kidney, testes and ovary	Na^+–dependent Cl^-/HCO_3^- exchanger, acid extruder	?		Inhibitor: DIDS	Chen et al., 2008; Burette et al., 2012
SLC4A10 (NCBE)	Brain	pH regulation (acid extrusion)	?	Higher seizure threshold, volume reduction of brain ventricles	Inhibitor: DIDS	Chen et al., 2008; Jacobs et al., 2008; Liu et al., 2011; Romero et al., 2013

TABLE 4 | SLC26 family of anion transporters.

SLC26	Expression	Function	Human disease	KO mouse	Pharmacology	References
SLC26A1 (SAT1)	Kidney, GI tract, liver, lung	SO_4^{2-}/Ox^{2-} exchanger, SO_4^{2-}/HCO_3^- exchanger		Oxalate urolithiasis, nephrocalcinosis, urinary sulfate wasting, hepatotoxicity		Xie et al., 2002; Soleimani and Xu, 2006; Soleimani, 2013
SLC26A2 (DTDST)	Kidney, GI tract, chondrocytes	Cl^-/SO_4^{2-} transporter, SO_4^{2-}/Ox^{2-} exchanger	Diastrophic dysplasia	Diastrophic dysplasia		Hästbacka et al., 1994; Soleimani and Xu, 2006 Soleimani, 2013
SLC26A3 (DRA)	GI tract, epididymis, enterocytes	Cl^-/HCO_3^- exchanger	Congenital chloride diarrhea	Congenital chloride diarrhea		Höglund et al., 1996; Soleimani, 2013
SLC26A4 (pendrin)	Kidney, inner ear, thyrocytes, lung	Cl^-/HCO_3^- exchanger	Pendred syndrome	Deafness, enlargement of the vestibular aqueduct		Reardon and Trembath, 1996; Everett et al., 1997; Kopp, 2000; Soleimani, 2013
SLC26A5 (prestin)	Cochlear hair cells	Cl^-/HCO_3^- exchanger	Deafness	Deafness		Liberman et al., 2002; Liu et al., 2003; Cheatham et al., 2004; Alper and Sharma, 2013
SLC26A6 (PAT1)	GI tract, kidney, cardiac myocytes	Cl^-/HCO_3^- exchanger, Cl^-/Ox^{2-} exchanger, Cl^-/formate exchanger	?			Soleimani, 2001; Mount and Romero, 2004; Alper et al., 2006; Aronson, 2006
SLC26A7	Brain, kidney, GI tract, lung	Cl^-/HCO_3^- exchanger, Cl^- channel	?	Locomotor impairment, gastric hypochlorhydria, distal renal tubular acidosis	Inhibitor: DIDS	Kim et al., 2005; Xu et al., 2006; Soleimani, 2013; Rahmati, 2015
SLC26A8	Male germ cells, kidney	Cl^-/Ox^{2-} exchanger, Cl^-/SO_4^{2-} exchanger	?	Male infertility		Touré et al., 2001; Lohi et al., 2002; Soleimani and Xu, 2006
SLC26A9	Stomach, lung, lower levels in kidney	Cl^-/HCO_3^- exchanger, Cl^- channel, Na^+/Cl^- cotransporter	?	Hypertension		Xu et al., 2005; Amlal et al., 2013; Soleimani, 2013
SLC26A11 (KBAT)	Brain, kidney, GI tract	Cl^- channel, Cl^-/HCO_3^- exchanger, volume control, pH regulation	?	Locomotor impairment	Inhibitor: GlyH-101, CFTRinh, DIDS (partial inhibition)	Vincourt et al., 2003; Rahmati et al., 2013, 2016; Rungta et al., 2015

different parts of the brain with various intensities, including cerebral cortex, hippocampus, olfactory bulb, and cerebellum. Cerebellar PCs show prominent expression of KBAT (Rahmati et al., 2013). At the subcellular level, KBAT was identified both in the cytoplasm and at the plasma membrane of PCs. Studies on HEK293 cells showed that KBAT can operate as a Cl^- channel that functionally interacts with H^+-ATPase. Transfection of cells with KBAT stimulated acid transport via H^+-ATPase and the cells with KBAT expression showed a more robust recovery from intracellular acidosis relative to mock transfected cells (Rahmati et al., 2013).

Studies on hippocampal and cortical pyramidal cells have reported the direct involvement of KBAT in cell death after cytotoxic edema (Rungta et al., 2015). Cytotoxic edema is one of the hallmark features of TBI and starts with an excessive Na^+ entry that depolarizes the membrane (Rungta et al., 2015). These processes activate the Cl^- influx through KBAT channels, which in turn causes cell swelling and cell death (Rungta et al., 2015). Inhibition of KBAT by utilizing siRNA-mediated knockdown of KBAT significantly prevents Cl^- influx and cell death after cytotoxic edema (Rungta et al., 2015). Rungta and colleagues showed that in their mouse model of TBI the recovery mechanism after increased cell volume was independent of NKCC1 and KCC2 activity as 100 μM bumetanide did not significantly affect the volume of swollen neurons.

In the cerebellum, recent data support the role of KBAT in intracellular Cl^- accumulation. Selective deletion of KBAT from PCs causes a significant reduction in $[Cl^-]_i$ and a more negative E_{Cl}. At the behavioral level, lack of KBAT in PCs causes deficits in locomotor activity (Rahmati et al., 2016). Considering the role of KBAT in neuronal Cl^- transport, as well as its involvement in cell swelling after cytotoxic edema, KBAT may provide a novel target for designing new therapeutic strategies for neurological conditions such as TBI.

4. CATION-CHLORIDE CO-TRANSPORTERS (SLC12 FAMILY)

The SLC12 family has been studied in greater detail compared to other Cl^- transporters. It is known as the cation-chloride cotransporter gene family (CCC), which contains 9 members (SLC12A1- SLC12A9). SLC12 isoforms (except A8 and A9) transport Cl^-, together with Na^+ and/or K^+ in an electroneutral manner (Hebert et al., 2004). In neurons studies have mostly focused on the roles of SLC12A2 (NKCC1) and SLC12A5 (KCC2)

TABLE 5 | SLC12 family of cation-chloride cotransporters.

SLC12	Expression	Function	Human disease	KO mouse	Pharmacology	References
SLC12A1 (NKCC2)	Kidney, gastrointestinal tract, pancreatic β-cells, induced in hypothalamo-neurohypophyseal system (HNS) by osmotic stress	$Na^+/K^+/Cl^-$ cotransporter involved in salt reabsorption	Bartter's syndrome type I	Sever hypotension, hypokalemia, hypercalcinuria, metabolic alkalosis	Inhibitors: bumetanide (10 μM), furosemide	Rocha and Kokko, 1973; Simon et al., 1996; Xue et al., 2009; Alshahrani and Di Fulvio, 2012b; Castrop and Schießl, 2014; Konopacka et al., 2015
SLC12A2 (NKCC1)	Broad	$Na^+/K^+/Cl^-$ cotransporter involved in regulation of $[Cl^-]_i$ and cell volume, regulation of E_{GABA} in neurons	Dehydration, vomiting, dilated cardiomyopathy, respiratory weakness, pancreatic insufficiency, missense mutation in a group of patients with schizophrenia, seizure-like episodes	Impaired sensory perception, deafness, infertility, hypotension, reduction of saliva production, normal intestinal absorption, enhanced insulin secretion and improved glucose tolerance	Inhibitors: bumetanide (10 μM), furosemide	Cherubini et al., 1991; Ben-Ari et al., 1997; Delpire et al., 1999, 2016; Dixon et al., 1999; Evans et al., 2000; Grubb et al., 2000; Pace et al., 2000; Sung et al., 2000; Alshahrani and Di Fulvio, 2012a; Flores et al., 2016; Merner et al., 2016
SLC12A3 (NCC)	Kidney, peripheral blood mononuclear cells, colon, spleen, placenta, small intestine, prostate	Na^+/Cl^- cotransporter involved in salt reabsorption	Gitelman syndrome	Hypotension, hypokalemia, hypercalcinuria, hypomagnesemia	Inhibitor: thiazide	Costanzo, 1985; Ellison et al., 1987; Chang et al., 1996; Abuladze et al., 1998; Arroyo et al., 2013
SLC12A4 (KCC1)	Broad	K^+/Cl^- cotransporter, involved in cell volume regulation	?	No phenotype is reported	Inhibitor: furosemide	Kanaka et al., 2001; Mikawa et al., 2002; Arroyo et al., 2013
SLC12A5 (KCC2)	Brain, pancreatic β-cells, adrenal chromaffin cells, cancer cells	K^+/Cl^- cotransporter, involved in regulation of $[Cl^-]_i$, neuronal excitability and cell volume, modulation of insulin secretion	Epilepsy, tumor invasion/metastasis	Complete KO: death conditional KO: increased $[Cl^-]_i$, positive shift of E_{GABA}, neuronal hyperexcitability, impaired motor performance, and motor learning	Inhibitors: bumetanide (100 μM), furosemide, VU0463271, VU 0240551, ML077 activator: CLP257	Williams et al., 1999; Ben-Ari, 2002; Song et al., 2002; Xie et al., 2003; Wei et al., 2011; Seja et al., 2012; Arroyo et al., 2013; Lavertu et al., 2013; Yu et al., 2014; Kahle et al., 2016; Kursan et al., 2017; Liu et al., 2017; Moore et al., 2017
SLC12A6 (KCC3)	Broad	K^+/Cl^- cotransporter, involved in cell volume regulation	Andermann syndrome (ACCPN), epilepsy?	Hypertension, progressive neurodegeneration, reduced seizure threshold, deafness	Inhibitor: furosemide	Pearson et al., 2001; Hebert et al., 2004; Seja et al., 2012

in regulation of inhibition through $GABA_AR$ activity (Takayama and Inoue, 2007; Seja et al., 2012; Kawakita et al., 2013). Earlier studies reported differential expression patterns for NKCC1 and KCC2 during development with higher NKCC1 expressions in immature brain and increased KCC2 levels in the adult brain (Plotkin et al., 1997; Lu et al., 1999; Rivera et al., 1999; Stein et al., 2004; Dzhala et al., 2005). According to these studies, the protein expression ratio of NKCC1 to KCC2 can explain the differences in $[Cl^-]_i$ and GABAergic signaling during development. However, recent findings suggest that NKCC1 and KCC2 cannot always explain the levels of $[Cl^-]_i$ (Glykys et al., 2014, 2017; Sedmak et al., 2016), because their activities as ion transporters are controlled by post-translational modifications, such as protein phosphorylation (Rinehart et al., 2009; Friedel et al., 2015).

SLC12 isoforms in the brain are also involved in cell volume regulation. Transport of Cl^- and cations through some CCCs is accompanied by the movement of water which can lead to neuronal swelling or shrinkage, unless other volume-regulated Cl^- transporters and channels are activated (MacAulay et al., 2004; Zeuthen, 2010; Jourdain et al., 2011; Glykys et al., 2017). Mutations in genes encoding for CCC isoforms result in various brain pathologies, like seizures, cerebral edema, neurodevelopmental deficits, and neuropathic pain. Pharmacological inhibitors of CCC functioning, like bumetanide and furosemide, which are well-known as loop diuretics, inhibit the CCCs both *in vitro* and *in vivo* (Dzhala et al., 2005, 2008). In low concentrations (2-10 μM) bumetanide specifically inhibits NKCC1 and exerts antiepileptic effects in human neonates (Kahle et al., 2009). In the cerebellum, four members of SLC12 family are expressed; these include SLC12A2 (NKCC1), SLC12A4 (KCC1), SLC12A5 (KCC2), and SLC12A6 (KCC3) (Kanaka et al., 2001; Mikawa et al., 2002). Here, we review the literature on NKCC1, KCC2, and KCC3, which all have been studied in the brain in more detail (see **Table 5** for neuronal and non-neuronal expression of SLC12 family).

SLC12A2 (NKCC1)

NKCC1, which is an electroneutral Na^+-K^+-$2Cl^-$ cotransporter, is one of the main Cl^- accumulator in neurons (Brumback and Staley, 2008). Genetically modified animal models lacking NKCC1 show severe phenotypes, including: deafness due to inner ear dysfunction, deficits of spermatocyte production that lead to complete infertility, hypotension, reduction in saliva production, and sensory perception impairment due to abnormal responses of the dorsal root ganglion neurons to GABA release (Delpire et al., 1999; Dixon et al., 1999; Evans et al., 2000; Pace et al., 2000; Sung et al., 2000). Studies have shown that NKCC1 is heavily expressed in brain cells, including cerebellar neurons and glia. These studies reported that the expression of NKCC1 gradually decreases in cerebellar neurons except for granule cells (GrCs), which show robust expression in both neonatal and adult stages of the brain (Hübner et al., 2001; Kanaka et al., 2001; Li et al., 2002; Mikawa et al., 2002). It has been suggested that strong expression of NKCC1 in mature GrCs causes higher $[Cl^-]_i$ compared to

other cerebellar neurons (Seja et al., 2012). NKCC1 is also repeatedly reported to be involved in cell volume control (Russell, 2000; Friedrich et al., 2006). Lowering $[Cl^-]_i$ and cell shrinkage stimulate NKCC1 ion transport activity, which is associated with increased levels of NKCC1 protein phosphorylation (Haas, 1994; Lytle, 1997).

SLC12A5 (KCC2)

KCC2 plays a crucial role in regulating cell volume as well as neuronal excitability (Payne et al., 2003; Kahle et al., 2015). Under normal physiological conditions the electrochemical balance dictates K^+-efflux, which supports exchange of one K^+ with one Cl^- by KCC2 and leads to the reduction of $[Cl^-]_i$ (**Figure 2**). Although KCC2-mediated ion transport occurs in normal isotonic conditions, cell swelling causes a 20-fold increase in its activity (Song et al., 2002). In the cerebellum, KCC2 is expressed in PCs, GrCs, MLIs, and CN (Haas, 1994; Williams et al., 1999; Mikawa et al., 2002). At the subcellular level, KCC2 is detected at the plasma membrane and it is localized at both cell body and dendrites of PCs (Seja et al., 2012). Gramicidin-perforated patch-clamp recordings of PCs from PC-specific KCC2 knockout mice revealed that KCC2 is the major Cl^- extruder of PCs (Seja et al., 2012). Studies on PC-specific and GrC-specific knockouts of KCC2 indicated that their $[Cl^-]_i$ was doubled in both PCs and GrCs. This significant increase in $[Cl^-]_i$ almost eliminated GABA-induced hyperpolarization in PCs without affecting their resting membrane potential. In GrCs, which have been shown to have a more positive E_{GABA} compared to RMP in normal physiological conditions (Seja et al., 2012), elevated $[Cl^-]_i$ caused higher excitability by depolarizing the resting membrane potential by ∼15 mV (Seja et al., 2012). Utilizing cerebellar specific behavioral tests, such as compensatory eye movements, Seja et al. (2012) showed that KCC2 deletion in PCs resulted in impairments in baseline motor performance, as well as motor learning. GrC-specific KCC2 knockout mice did not show prominent deficits in their baseline motor performance, while they were significantly impaired in motor learning (for details see Seja et al., 2012).

SLC12A6 (KCC3)

KCC3 is a widespread K^+-Cl^- cotransporter, which particularly shows significant expression in brain and spinal cord (Pearson et al., 2001). In the cerebellum, KCC3 is expressed in cerebellar PCs (Pearson et al., 2001; Boettger et al., 2003). Conventional KCC3 knockout mice revealed important roles at cellular and behavioral levels. Deletion of KCC3 increased the $[Cl^-]_i$ in PCs and resulted in severe motor abnormalities. The knockout mice also showed hypertension, progressive neurodegeneration and deafness, as well as reduced seizure threshold (Boettger et al., 2003). However, later studies on PC-specific KCC3-KO mutant mice could not detect any difference with control mice neither at the cellular nor behavioral level (Seja et al., 2012). It has been proposed that KCC3 mostly functions as a volume regulator in mature neurons, while in immature neurons it may participate in modulation of $[Cl^-]_i$ and network development (Seja et al., 2012).

5. LIGAND-GATED CL^- CHANNELS

Hyperpolarizing inhibition was discovered in 1951 by John C. Eccles and his colleagues (Brock et al., 1951). Their work on identification of ionic mechanisms underlying the generation of inhibitory postsynaptic potentials (IPSPs) and the activity of ligand-gated Cl^- channels questioned the "passive distribution dogma" of Cl^-. Ever since, researchers have been investigating the underlying mechanisms of $[Cl^-]_i$ regulation and neuronal inhibition in various parts of the brain (**Table 6**).

5.1. GABA-Activated Cl^- Channels

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and plays vital roles in the development, migration, and assembly of neurons to create functional networks (Mcbain and Fisahn, 2001; Whittington and Traub, 2003; Farrant and Kaila, 2007). In the CNS, GABA receptors appear as ionotropic $GABA_A$ receptors ($GABA_A$Rs) and metabotropic $GABA_B$ receptors; the latter do not directly gate anions and therefore are not considered in this review.

TABLE 6 | Ligand-gated chloride channels.

Channel	Expression	Function	Human disease	KO mouse	Pharmacology	References
$GABA_A$R	Nervous system	Inhibitory synaptic transmission in the brain, neuronal excitability and development	Epilepsy, movement disorders, cognitive disorders, autism, anxiety disorders, schizophrenia, sleep disorders, mood disorders	Epilepsy, movement disorders, impaired motor learning and cognition	Agonists: benzodiazepines, barbiturates, zolpidem, muscimol antagonists: bicuculline, picrotoxin, Cu^{2+} (blocks tonic inhibition)	Kaila, 1994; Möhler, 2006; Wisden et al., 2009; Gonzalez-Burgos et al., 2011; Braat and Kooy, 2015
GlyR	Nervous system	Inhibitory synaptic transmission in the central nervous system, neuronal excitability and development	Startle disease, autism	Natural occurring mutation: startle disease	Agonists: Taurine, α-L-alanine, L-serine, low concentration of Zn^{2+}, antagonists: strychnine, high concentration of Zn^{2+}	Curtis et al., 1968; Werman et al., 1968; Lynch, 2004; Burgos et al., 2016; Ito, 2016
SLC1A4 (EAAT4)	Nervous system	Glutamate/Na^+/Cl^- transport, involved in neuronal excitability and development	Neurodegenerative disorders, stroke	Down-regulation of EAAT4 leads to PC hyperexcitability	Agonists: TBOA, L-α-aminoadipate, T3MG, Zn^{2+} (selective blocker of Cl^- conductance)	(Fairman et al., 1995; Nagao et al., 1997; Fairman and Amara, 1999)

$GABA_A$Rs are anion selective channels that are gated upon binding of GABA, permeable to Cl^- and HCO_3^- and mediate a fast postsynaptic current. Due to $GABA_A$Rs' five times higher permeability to Cl^- than HCO_3^- and four times higher concentration of extracellular Cl^- compared to HCO_3^-, $[Cl^-]_i$ is considered to be the main determinant of the direction of current through $GABA_A$Rs (Staley, 2011). In other words, $[Cl^-]_i$ dictates the GABAergic reversal potential (E_{GABA}). However, the permeability to HCO_3^- functions as a depolarizing current through $GABA_A$Rs. Therefore, the E_{GABA} differs from E_{Cl} to more positive values (Kaila and Voipio, 1987).

$GABA_A$ receptors are pentameric proteins composed of different subunits (Carlson et al., 1998). It is known that in human there are six α subunits, three β subunits, three γ subunits, three ρ subunits, and one ε, δ, θ, and π subunit (Sigel and Steinmann, 2012). This reflects the large diversity of subunit assembly, which is further increased by alternative splicing. The subunit composition, which is shown to be important for the kinetics and pharmacological properties of the receptor, changes during development (Succol et al., 2012). Whereas immature GABAergic synapses contain high expression levels of the α3 subunit, the adult isoform of $GABA_A$ receptor mainly contains α1, β2, and γ2 subunits (Ortinski et al., 2004). In the adult cerebellum, whole-cell recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) in PCs and GrCs revealed differences between the shapes and decay times of the recorded currents. Purkinje cells typically express a combination of α1-, β2, or β3- and γ2-subunits, which mediates sIPSCs with a single fast exponential decay time constant, whereas GrCs, which specifically express the α6 subunit, show sIPSCs with the sum of fast and slow exponential curves, indicating that GrCs express both fast- and slow-mediated receptor compositions (Wisden, 1995). The variation in the expression of subunits does not only differ depending on the cell type, but also on subcellular location (e.g., soma vs. dendrites) (Wisden, 1995). To estimate the impact of local $GABA_A$Rs on the membrane potential and $[Cl^-]_i$, one should consider the location together with the composition of subunits, which also determines the conductance level of the $GABA_A$Rs (Porter et al., 1992; Angelotti and Macdonald, 1993). Studies on HEK293 cells showed that α1β2γ2-containing $GABA_A$Rs, which is the composition expressed in mature cerebellar PCs, have the highest conductance of 30 pS (Verdoorn et al., 1990).

Throughout the olivocerebellar system all cell-types appear to express $GABA_A$Rs where they not only gate phasic inhibition, which is fast and lasts for milliseconds, but also gate tonic inhibition. The former type of synaptic inhibition is gated by synaptic $GABA_A$Rs and the latter mostly by extrasynaptic $GABA_A$Rs (Wisden, 1995; Farrant and Nusser, 2005; Sigel and Steinmann, 2012). The targeting of $GABA_A$Rs to synaptic or extrasynaptic compartments is determined by the presence of specific subunits (Pirker et al., 2000). For instance, the γ2 subunit is primarily expressed at synaptic sites, whereas the δ subunit is mostly present at extrasynaptic locations (Schweizer et al., 2003; Brickley and Mody, 2012; Brickley et al., 2013). In addition to phasic and tonic inhibition, spillover inhibition also occurs in cerebellar cortex, a phenomenon that is moderated by GABA spilling out of the synaptic cleft. For example, high affinity $GABA_A$Rs expressed on GrCs can sense low concentrations of GABA spilling out of their synapses with GoCs (Rossi and Hamann, 1998). The impact of GABA spillover in cerebellar cortex has also been demonstrated at excitatory terminals within the cerebellar glomerulus (Mitchell and Silver, 2000). In olivocerebellar system, some of the subunits of $GABA_A$Rs show ubiquitous expression, whereas others represent a restricted expression pattern, such as the α6 subunit, which is only expressed in cerebellar granule cells (Carlson et al., 1998; Sieghart and Sperk, 2002). Inferior olive neurons abundantly express α2, α4, and γ1-subunits, while α1, α3, β2, β3, and γ2 are detected at lower levels (Laurie et al., 1992). Expression of the α3 subunit appears to be restricted to the soma of olivary neurons and mediate a slow postsynaptic response to GABA (Bengtsson and Hesslow, 2006).

$GABA_A$Rs are involved in various brain disorders, including epilepsy, movement disabilities, cognitive disorders, anxiety disorders, mood disorders, schizophrenia, autism, and sleep disorders (Gonzalez-Burgos et al., 2011). Various synaptic connections in the olivocerebellar network are GABAergic and through the inhibition of postsynaptic neurons can not only dampen action potential firing, but also drive timed action potential firing in groups of neurons with strong pace-making activity, such as PCs and CNs and thereby promote synchronous activity (Wulff et al., 2009; Hoebeek et al., 2010; Buzsáki and Wang, 2012; Person and Raman, 2012). In addition, the direct projection from the cerebellum to the IO is GABAergic, which is crucial for modulating activity patterns in the olivo-cerebellar-olivary loop (Bengtsson and Hesslow, 2006). Several studies have utilized the Cre-loxP system to create mouse models with specific deletion of GABAergic synapses in subsets of cells. For instance, deletion of the γ2 subunit in PCs (PC-γ2 KO) leads to disruption of the receptor targeting to the postsynaptic membrane and selective removal of synaptic $GABA_A$Rs from PCs (Wulff et al., 2009). These studies have shown that deletion of GABAergic input to PCs by removing $GABA_A$Rs alters the temporal pattern of PC activity by affecting the regularity of both spontaneous and parallel fiber-evoked simple spike firing. *In vivo* extracellular recordings of PCs from PC-γ2 mice also showed higher simple spike firing regularity in the flocculus during compensatory eye movement and spontaneous behavior (Wulff et al., 2009), indicating that GABAergic inputs from MLIs to PCs may form the main source of irregularity in PC firing pattern (Häusser and Clark, 1997). Compensatory eye movement experiments on PC-γ2 mice revealed severe motor learning deficits and supported the importance of GABAergic signaling and feed-forward inhibition in cerebellar learning (Wulff et al., 2009). Surprisingly, PC-γ2 did not show ataxia, but only mild gait abnormalities (Veloz et al., 2015). In order to understand whether genetic compensatory mechanisms were involved in saving these mice from strong baseline motor deficits, Wisden and colleagues published a study in 2009 where they manipulated $GABA_A$Rs by intraperitoneal injection of zolpidem (Wisden et al., 2009). To specifically study the MLI-PC synapse, they developed genetically modified mouse models, which were selectively sensitive to rapid manipulation of $GABA_A$R modulator zolpidem only at MLI-PC

synapse (PC-γ2-swap mice). PC-γ2-swap mice showed severe motor abnormalities, highlighting the crucial role of MLI-PC synapse-mediated inhibition in motor control (Wisden et al., 2009). Studies have also examined the importance of GABAergic input to GrCs by eliminating Golgi cells; this manipulation caused overexcitation of GrCs and resulted in sever ataxia (Watanabe et al., 1998).

In the IO $GABA_A$Rs modulate the strength of the inhibitory response to the afferent input from CN. Apart from classical hyperpolarizing effects, the CN-IO GABAergic synapses are suggested to be important for regulation of neuronal coupling in the olive (De Zeeuw et al., 2011). Thereby GABA-mediated inputs to the IO are not only affecting the excitability of olivary neurons, but also the local oscillations (Bazzigaluppi et al., 2012; De Gruijl et al., 2012). Both these mechanisms have profound effects on cerebellar PC and CN firing by means of CF innervation (De Zeeuw et al., 2011). For instance, it is shown that CFs regulate the synaptic plasticity at the level of PF-PC, as well as MLI-PC synapses by inducing long-term depression (LTD) and rebound potentiation (RP), respectively (Kano et al., 1992). Therefore, the activity level of olivary neurons can induce long-term changes at different cerebellar synapses (Kano et al., 1992; Gao et al., 2012; Ito, 2012; Hirano and Kawaguchi, 2014).

5.2. Glycine-Activated Cl^- Channels

The glycine receptors (GlyRs) function as ligand-gated Cl^- channels. Similar to $GABA_A$Rs, GlyRs mediate excitatory or inhibitory responses by promoting the efflux or influx of Cl^-, respectively (Curtis et al., 1968; Werman et al., 1968)—the polarity is determined by the membrane potential and E_{Cl}. GlyRs are found throughout the central nervous system, with noticeable densities in the spinal cord, cerebellum, hippocampus, amygdala, hypothalamus, substantia nigra, cochlear nuclei, superior olivary complex, and trapezoid body (Zarbin et al., 1981; Flint et al., 1998; Ye et al., 1999; Mccool and Botting, 2000; Chattipakorn and McMahon, 2002; Gaiarsa et al., 2002; Mangin et al., 2002; Ye, 2007; Planells-Cases and Jentsch, 2009). GlyRs undergo developmental changes in their subunit composition. They mostly contain the α2 subunit at early stages of development, whereas the adult receptor contains 2α1/3β subunits (Becker et al., 1988; Lynch, 2004; Grudzinska et al., 2005). These subunits have different localizations and expression intensities. In the cerebellar cortex, GlyRs are labeled on the dendrites of Golgi cells where they receive inhibitory input from Lugaro cells (Dumoulin et al., 2001). In the CN, there is a small group of glycinergic, or mixed glycinergic/GABAergic interneurons, which locally innervate principal CN neurons (Chan-Palay, 1977; Zeeuw and Berrebi, 1995). Studies by Husson and colleagues identified predominant expression of GlyRs on principal output CN neurons, where they receive inhibitory inputs from local CN interneurons (Husson et al., 2014). They showed that at these synapses GlyRs mediate Cl^- currents and participate in inhibition of CN principal neurons, alike the GABAergic input from PCs. In addition, there is a subgroup of glycinergic projection neurons in the CN that adjusts the impact of glutamatergic inputs and facilitates vestibulocerebellar function (Bagnall et al., 2009).

Mutations in GlyRs have been identified in humans and cause autosomal dominant and recessive hyperekplexia or startle disease, which is characterized by pronounced and exaggerated responses to tactile or acoustic stimuli and hypertonia (Shiang et al., 1993). Natural occurring murine startle disease has been reported and shows similar phenotypes, such as reduced glycine sensitivity or reduced membrane expression of GlyRs. The specific impact of GlyRs in cerebellar activity has not been studied in detail yet. Most GlyRs seem to operate only during a limited developmental window and may contribute to the establishment of synaptic connections. In the developing cerebellum, *in vitro* whole-cell patch-clamp recordings of PCs from rats on postnatal days 3–10 (P3-P10) revealed a significant increase in frequencies of both excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) upon application of 100 μM glycine (Kawa, 2003; Ye, 2007). These glycine-evoked synaptic events were abolished by application of strychnine (1 μM), a specific blocker of the GlyRs (Kawa, 2003). Thus, ionotropic GlyRs may be transiently expressed in the developing cerebellum and play important roles in maturation and organization of cerebellar circuits (Kawa, 2003; Ye, 2007).

5.3. Glutamate-Activated Cl^- Channel (EAAT4)

Excitatory amino acid transporters (EAATs) are known to play a crucial role in terminating glutamatergic transmission by uptake of glutamate from the synaptic cleft. This function is necessary to prevent glutamate receptor overstimulation (Robinson and Dowd, 1996; Dehnes et al., 1998). In addition, EAATs can function as glutamate-activated, Na^+ dependent Cl^- channels (Fairman et al., 1995; Wadiche et al., 1995; Nagao et al., 1997). They belong to the solute carrier family 1 (SLC1), which contains five members: (EAAT1-EAAT5). EAAT4 is highly expressed in the cerebellum with an uneven parasagittal zonal distribution, which is very similar to that of Zebrin II (aldolase C) (Brochu et al., 1990; Dehnes et al., 1998). *In situ* hybridization, immunohistological studies and electron microscopy have revealed that EAAT4 is a postsynaptic transporter with predominant expression on PC spines where they receive inputs from PFs and CFs (Takahashi et al., 1996; Nagao et al., 1997; Otis et al., 1997). Other studies have reported the extrasynaptic expression of EAAT4 on PC spines, where it restricts glutamate spillover to neighboring synapses (Tanaka et al., 1997). Electrophysiological studies of PCs indicate that after glutamate release and high frequency action potential firing, EAAT4 increases its Cl^- transport, which may serve as an extra force for limiting excessive PC firing (Dehnes et al., 1998). Given the preponderance of EAAT4 in the zebrin-positive zones, which probably all require a low baseline firing frequency of simple spikes so as to allow ample enhancement during motor learning (Zhou et al., 2014), this prevention fits in perfectly with ongoing hypotheses on the roles of zonally distributed forms of long-term postsynaptic potentiation and depression (De Zeeuw and Ten Brinke, 2015).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

While the roles of Cl^- channels and transporters are established and proven to be vital in various organs of the body, their impact on neuronal activity requires further investigations. Neurons express a rich set of plasma membrane Cl^- channels and transporters, which belong to various protein families and have different modes of activation. The roles and regulations of many of these transporters and channels in the brain remain to be elucidated. As we have discussed here, Cl^- transporting proteins show distinct expression patterns in the brain and are activated through different intra- and extracellular signaling pathways to establish and maintain the $[Cl^-]_i$. Investigating how Cl^- channels and transporters function and how they interact to set the $[Cl^-]_i$ will help develop new strategies for treatment of various neurological conditions linked to aberrant Cl^- homeostasis. The recent advances in fluorescent Cl^- indicators have considerably helped with understanding the variability in $[Cl^-]_i$ and GABAergic signaling in different brain regions. Utilizing these Cl^- indicators, the impact of Cl^- transporting proteins and other Cl^- regulatory mechanisms on $[Cl^-]_i$ can be simultaneously visualized for hundreds of cells. However, the interpretation of the physiological and pathophysiological modifications in $[Cl^-]_i$ is not easy, because the intracellular Cl^- dynamics are tightly intermingled with multiple cellular mechanisms, such as pH modifications and membrane potential regulation. Therefore, Cl^- dynamics need to be investigated by combining Cl^- measurements with pH imaging and electrophysiological recordings. In addition, the significance of deletions or mutations of Cl^- transporters and channels on regulating $[Cl^-]_i$ and neuronal activity needs to be analyzed while taking developmental compensations into account. Recent advances of genetic sequencing techniques such as RNA sequencing analysis have now made it possible to take a further step in investigating the impacts of genetic compensations. Together, these approaches will ultimately shed light on the complex interactions of various ionic channels and transporters and their up and/or down regulations during development and adulthood. In this manuscript, we reviewed the main Cl^- channels and transporters currently known for the olivocerebellar system, which is implicated in various movement disorders and probably also in neurological diseases like epilepsy.

AUTHOR CONTRIBUTIONS

NR, FH, SP, and CD contributed to writing the review paper.

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