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Review

Pharmacological and functional implications of developmentally-regulated changes in $GABA_A$ receptor subunit expression in the cerebellum

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Abstract

The cerebellum undergoes many morphological, pharmacological, and electrophysiological changes during the first 3 weeks of postnatal development. The purpose of this review is to present the most up to date synopsis of the pharmacological and functional changes in, γ-aminobutyric acid (GABA) type A receptors during this time of cerebellar maturation. Since most of the diversity in cerebellar, $GABA_A$ receptor pharmacology lies within the granule cell layer, research groups have focused on this area of the cerebellum to study the developmental changes in $GABA_A$ receptor subunit expression and the neurodifferentiating factors involved in regulating this expression. Thus, it is important to note that developmental changes in $GABA_A$ receptor composition and its corresponding pharmacology will be essential for determining the type of GABA-mediated transmission that occurs between neuronal contacts in the neonatal and subsequently in the mature cerebellum.

Keywords: Benzodiazepine-insensitive binding; Neurodifferentiation; Cerebellar granule cell; α_6 subunit; GABA (γ-aminobutyric acid) affinity; Cultured neuron

1. Introduction

1.1. Cellular development in the cerebellum

The cerebellum consists of a limited number of neuronal cell types, the development of which is quite different. The postnatal development in the rat of Purkinje cells, climbing fibers, granule cells, parallel fibers, basket cells, stellate cells, and Golgi neurons has been outlined in detail by Altman (1972). The Purkinje cells form their dendritic trees during a two week period from day 7 to day 21. During this period, granule cells migrate from the external granule layer to the internal granule layer; parallel fibers are formed; and synaptic contacts are established between these and the Purkinje cell dendritic trees. It should be noted that these contacts are formed relatively late. It appears that the maturation of the Purkinje cell dendrites is dependent upon contacts with climbing fibers rather than the parallel fibers (Rakic and Sidman, 1973).

The granule cells, which constitute the vast majority of neurons in the cerebellum, undergo cell division until postnatal days 7–9 at which time their characteristic differentiation process is initiated. This process involves sprouting to generate parallel fibers and subsequent outgrowth of the fibers leading to migration of the cell body to the internal granule layer, a process guided by Bergman glia fibers already formed at postnatal day 2 (Eccles, 1970; Rakic, 1973; Altman, 1975; Komuro and Rakic, 1998).

Due to the fact that granule cells outnumber the other types of neurons in the cerebellum by a thousand fold, it is quite easy to establish an in vitro cell culture system to study the development of granule cells (Lasher and Zagon, 1972; Messer, 1977; Schousboe et al., 1989). Such cultures are generally initiated using dissociated cells from 7- to 8-day-old mice or rats, since this time point corresponds to the postnatal age at which proliferation stops (Hertz et al., 1985). In order for these immature neurons or neuroblasts to develop processes and functionally active nerve endings in culture, it is essential that the cells receive a depolarizing stimulus during the first few days in culture, either by exposure to 25 mM KCl or to low micromolar concentrations of *N*-methyl-D-aspartate (i.e., NMDA) or kainate

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(Lasher and Zagon, 1972; Gallo et al., 1987; Balazs et al., 1988, 1990; Peng et al., 1991; Damgaard et al., 1996). Treatment of the cultures with 50 μM kainate during the first week in vitro additionally leads to selective elimination of stellate, basket, and Golgi cells (Drejer and Schousboe, 1989; Schousboe and Pasantes-Morales, 1989; Damgaard et al., 1996). Addition of the antimitotic agent cytosine arabinoside (20 μM) prevents proliferation of astroblasts, which leads to a cell culture consisting of 90–95% granule neurons, 5–10% astrocytes and no other types of neurons (Messer, 1977; Larsson et al., 1985). Cerebellar granule cells are characterized as glutamatergic neurons (Young et al., 1974), and Golgi, stellate, and basket cells which contain γ-aminobutyric acid (GABA) are termed, GABAergic (Fagg and Foster, 1983). Therefore, this procedure leads to a pure preparation of glutamatergic neurons with no contamination of GABAergic cells, which would otherwise exhibit a pronounced release of GABA (Pearce et al., 1981; Damgaard et al., 1996).

Using this culture system, it has been possible to demonstrate that exposure of the granule cells to GABA or γ-aminobutyric acid$_A$ ($GABA_A$) receptor agonists promotes the morphological differentiation of these neurons as evidenced by stimulation of neurite formation and an increase in the cytoplasmic density of organelles involved in protein synthesis and processing (Hansen et al., 1984, 1987; Meier et al., 1985). A similar neurotrophic or neurodifferentiative action of GABA and $GABA_A$ receptor agonists has been seen also in other cell culture systems and in the brain in vivo (Wolff et al., 1979; Spoerri and Wolff, 1981; Eins et al., 1983; Meier et al., 1987a,b).

2. Developmental changes in cerebellar $GABA_A$ receptors

The $GABA_A$ receptor belongs to a superfamily of ligand-gated ion channels, which is typified by the nicotinic acetylcholine receptor. In the case of the $GABA_A$ receptor, a chloride ion channel is formed from a multioligomeric structure, and it is allosterically gated by GABA and other naturally occurring and synthetic agonists. Molecular cloning has identified 19 distinct $GABA_A$ receptor proteins, which can be grouped into six subunit families (i.e., α, β, γ, δ, ε, ρ). In addition to ρ homomeric channels in the retina (Cutting et al., 1991), the stoichiometry of native $GABA_A$ receptors in the central nervous system (CNS) is believed to have a five subunit configuration, containing two α subunits, two β subunits, and a γ or a δ or an ε (Chang et al., 1996; Davies et al., 1997; Tretter et al., 1997). The following sections will discuss how affinity, pharmacology, and chloride gating of $GABA_A$ receptors in the cerebellum change during postnatal development, and how these aspects may relate to specific $GABA_A$ receptor subtypes.

2.1. GABA affinity

Studies of ligand binding to $GABA_A$ receptors using thoroughly washed synaptosomal membranes generally reveal multiple binding sites with different affinities for the ligand (Wang et al., 1979; Olsen et al., 1981; Meier and Schousboe, 1982; Falch and Krogsgaard-Larsen, 1982). During the ontogenetic development of rat cerebellum, these binding sites for GABA follow different time courses. The high affinity sites (i.e., K_d for GABA around 10 nM) are fully developed at 2 weeks postnatally, whereas a population of low affinity sites (i.e., K_d around 500 nM) develops much later not reaching the maximum number until 2 months after birth (Meier et al., 1983). Compelling evidence has been presented that this transition is influenced by the availability of a GABA signal activating high affinity receptors during very early development (Meier et al., 1983, 1984; Madtes and Redburn, 1983a,b; Belhage et al., 1988). Other studies have similarly concluded that the expression of different $GABA_A$ receptor subunits is differentially regulated by developmental cues (Killisch et al., 1991; Beattie and Siegel, 1993).

A detailed study of the developmental profile of α_{1-6}, β_{1-3}, γ_{1-3} and δ subunits of $GABA_A$ receptors in the cerebellum has revealed some interesting transitions of subunit expression which may correlate with the change between high affinity receptors and high plus low affinity receptors (Laurie et al., 1992b). Around postnatal day 6, there is a significant upregulation in the mRNA expression of the α_1, α_6, β_2, β_3, γ_2 and δ subunits while the mRNA expression of the α_2, α_3 and β_1 subunits is decreased. It should be noted in this context that the expression of the α_1, α_6, β_2 and β_3 subunits seems to be regulated by $GABA_A$ receptor agonists which stimulate the expression at the mRNA level and the protein level (Hansen et al., 1991; Kim et al., 1993; Elster et al., 1995; Carlson et al., 1997). How exactly the change in affinity of the receptor complexes, formed during this period, is correlated to the relative abundance of these subunits is presently unknown. Several reports, however, have indicated that specific subunits confer differences in binding affinity for agonists, such as muscimol and THIP (i.e., 4,5,6,7-tetrahydroisoxazolo[5,4,*c*]pyridin-3-ol), to $GABA_A$ receptor complexes (Bureau and Olsen, 1990, 1993; Ebert et al., 1997). Specifically, the α_6 subunit demonstrates the highest affinity for $GABA_A$ receptor agonists (i.e., GABA, muscimol, THIP) as compared to the other five α subunits (Ebert et al., 1997). Likewise, replacing a γ for a δ subunit increases the affinity for agonists (Shivers et al., 1989). Thus, because the granule cell layer selectively displays the largest number of high affinity binding sites for $GABA_A$ receptor agonists in the CNS, it appears that the presence of these high affinity sites are most likely accounted by $GABA_A$ receptors containing the α_6 and/or δ subunits (Olsen et al., 1990; Benke et al., 1991b; Laurie et al., 1992a).

2.2. Benzodiazepine pharmacology in the developing cerebellum

Benzodiazepines have laid the groundwork for studies of $GABA_A$ receptor heterogeneity. High and low affinity binding sites for the triazolopyridazine, Cl 218872, have divided $GABA_A$ receptors into Type 1 (high affinity) and Type 2 (low affinity) subgroups (Squires et al., 1979; Young et al., 1981; Olsen et al., 1990). The inverse agonist, ethyl β-carboline-3-carboxylate, has also been used to characterize $GABA_A$ receptors into the subdivisions of high and low affinity benzodiazepine binding sites, using the nomenclature of BZ_1 and BZ_2, respectively (Braestrup and Nielsen, 1981). Since the original cloning of the $GABA_A$ receptor in the late 1980s (Schofield et al., 1987), additional pharmacological heterogeneity has been identified, and molecular cloning has revealed an extensive complexity of receptor composition with the identification of six different subunit families (α_{1-6}, β_{1-4}, γ_{1-4}, δ, ρ_{1-3}, ε) (Macdonald and Olsen, 1994; Stephenson, 1995; Ogurusu and Shingai, 1996; Davies et al., 1997). $GABA_A$ receptors that confer benzodiazepine modulation will be composed of at least an α, β and γ subunit. The type of β subunit in the complex does not have any significant impact on benzodiazepine affinity (Pritchett et al., 1989a; Hadingham et al., 1993b; Wafford et al., 1994); however, it is essential for the assembly of pentameric $GABA_A$ receptors (Connolly et al., 1996). On the contrary, both the α and γ subunits are important for benzodiazepine modulation. The α subunit determines both the affinity and efficacy of benzodiazepine binding, and the presence of the γ subunit complements the α subunit for the manifestation of benzodiazepine binding (Pritchett et al., 1989a,b; Wafford et al., 1993a,b; Hadingham et al., 1993a). It should be noted that amino acid residues on both the α and γ subunits are implicated for binding of benzodiazepines (reviewed in Smith and Olsen, 1995). Recombinant expression of the six cloned α subunits with β and γ subunits in a suitable combination shows that α_1 supports Type $1/BZ_1$ binding, and that α_2, α_3 and α_5 subunits represent the Type $2/BZ_2$ subpopulation (Pritchett et al., 1989a; Pritchett and Seeburg, 1990; Hadingham et al., 1993a; Wafford et al., 1993a). $GABA_A$ receptors containing the α_4 or α_6 subunits are insensitive to most types of benzodiazepines, such as diazepam and flunitrazepam (Lüddens et al., 1990; Wisden et al., 1991; Wieland et al., 1992). Interestingly, it is only a single histidine residue in the α_1 subunit, that is replaced by an arginine in the α_6 subunit, which confers benzodiazepine sensitivity (Wieland et al., 1992).

In granule cells from adult cerebellum high concentrations of α_1, α_6, β_2, β_3, γ_2 and δ mRNAs are found in comparison to low concentrations of the mRNAs for the α_4, β_1, and γ_3 subunits (Laurie et al., 1992a). Immunocytochemical studies have likewise shown a dense population of α_1, α_6, $\beta_{2/3}$ and δ subunits (Richards et al., 1986, 1987; De Blas et al., 1988; Somogyi et al., 1989; Benke et al., 1991a; Zimprich et al., 1991; Thompson et al., 1992; Gutiérrez et al., 1996; Khan et al., 1996; Nusser et al., 1995, 1996) and a moderate level of γ_2 immunoreactivity in the granule cell layer (Benke et al., 1991b; Gutiérrez et al., 1994). Moreover, the α_6 subunit has a unique distribution in the brain, in which it is almost exclusively found in cerebellar granule cells (Lüddens et al., 1990; Laurie et al., 1992a; Thompson et al., 1992). The imidazobenzodiazepine, ethyl-8-azido-6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5*a*]-[1,4]benzodiazepine-3-carboxylate (i.e., Ro 15-4513; sarmazenil) is one of the few known benzodiazepines that has a high affinity to benzodiazepine-insensitive $GABA_A$ receptors. This pharmacological agent has been useful for studying the ratio between benzodiazepine-sensitive and benzodiazepine-insensitive $GABA_A$ receptors. The benzodiazepine-sensitive/benzodiazepine-insensitive ratio of [^{3}H]Ro 15-4513 binding sites in the cerebellum is approximately 3:1 (Sieghart et al., 1987; Malminiemi and Korpi, 1989; Lüddens et al., 1990; Turner et al., 1991; Khan et al., 1993, 1994, 1996). Furthermore, about 50% of [^{3}H]Ro 15-4513 binding in the granule cell layer is not displaceable by benzodiazepines, meaning that they are benzodiazepine-insensitive (Malminiemi and Korpi, 1989; Carlson et al., 1997). Another pharmacological agent used for identifying specifically α_6-containing $GABA_A$ receptors is the loop diuretic, furosemide (Korpi et al., 1995). Quantitative autoradiography has shown that furosemide antagonism of GABA binding is localized exclusively to cerebellar granule cells (Korpi et al., 1995).

2.2.1. *In vivo* studies

The structural along with the pharmacological characteristics of $GABA_A$ receptors undergo changes in the developing cerebellum. There is a transient increase in the production of almost all $GABA_A$ receptor transcripts between embryonic day 19 to postnatal day 6 (Laurie et al., 1992b), which is also reflected in a transient heterogeneity in benzodiazepine binding, e.g., both Type $1/BZ_1$ and Type $2/BZ_2$ binding (Chisholm et al., 1983; Sieghart, 1986). An upregulation in the expression of α_1, β_2, β_3 and γ_2 $GABA_A$ receptor mRNAs in early postnatal granule cells (Laurie et al., 1992b), correlates with an increase in GABA binding and Type 1 benzodiazepine binding (Coyle and Enna, 1976; Candy and Martin, 1979; Palacios and Kuhar, 1982; Chisholm et al., 1983; Squires et al., 1990; Zdilar et al., 1991). During this same time, the α_6 subunit mRNA is not detected in proliferating granule cells (Laurie et al., 1992b). This finding agrees with benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding appearing after postnatal day 6 (Korpi et al., 1993). Korpi et al. (1993) have observed that the density of [^{3}H]Ro 15-4513 binding sites increased significantly with age, without any changes in the affinity for the ligand. The appearance of benzodiazepine-insensitive binding at the time when cerebellar

granule cells start to mature has been confirmed by autoradiography, and this study demonstrated that the binding was confined to the granule cell layer (Korpi et al., 1993).

2.2.2. In vitro studies

As compared to in vivo studies, the in vitro development of primary, cultured granule cells has shown a similar pattern of developmental changes in benzodiazepine pharmacology and expression of $GABA_A$ receptor subunits. Thompson and Stephenson (1994) have shown that the total binding of [^{3}H]Ro 15-4513 in cultures of cerebellar granule cells, made from 7- to 8-day-old rats, increases three fold from 1 to 9 days in vitro. Looking at the percent of benzodiazepine-sensitive and benzodiazepine-insensitive binding of total [^{3}H]Ro 15-4513 binding, there is a time-dependent enrichment, up to a maximum of 74%, for benzodiazepine-insensitive binding as compared to benzodiazepine-sensitive binding that does not change (Thompson and Stephenson, 1994). The binding data correlates well with an increased expression of the α_6 subunit from 3–5 days in vitro. Although benzodiazepine-sensitive binding did not change over time, the amount of α_1 protein continued to increase from 1–9 days in vitro (Thompson and Stephenson, 1994). An increase of α_1 mRNA in cultured cerebellar granule cells also grown from postnatal day 8 rats has been observed from 1–20 days in vitro (Mathews et al., 1994). Likewise, this group showed a relatively larger increase in α_6 mRNA level compared to α_1 mRNA, demonstrated as a nine fold increase for the α_6/γ_2 ratio compared to a three fold increase for the ratio between α_1/γ_2. Taken together, if α_1 mRNA and protein continues to increase over postnatal development, why does benzodiazepine-sensitive binding not follow a parallel pattern, too?

2.2.3. Molecular determinants of benzodiazepine pharmacology in the developing cerebellum

A possible reason for the stagnation of benzodiazepine-sensitive [^{3}H]Ro 15-4513 binding in early postnatal cultured cerebellar granule cells could be explained by the α_1 and α_6 subunits coexisting in the same receptor complex. Immunogold labeling of $GABA_A$ receptor subunits and visualization at the electron microscopic level have shown that α_1 and α_6 appear to co-localize in synapses between GABAergic Golgi neurons and granule cell dendrites, as well as at extrasynaptic sites on dendritic and somatic membranes (Nusser et al., 1996). Additionally, single cell reverse-transcriptase, polymerase chain reaction has shown that α_1 and α_6 mRNAs can be found in the same granule cell (Santi et al., 1994). Although the majority of immunoprecipitation studies support the coassembly of the two subunits in the same receptor complex (Lüddens et al., 1991; Pollard et al., 1993, 1995; Khan et al., 1994, 1996), one group has demonstrated that these two subunits do not coassemble into the same $GABA_A$ receptor (Quirk et al., 1994). Nevertheless, from one of the immunoprecipitation studies, it was reported that 39% of benzodiazepine-insensitive $GABA_A$ receptors in the cerebellum formed a pentamer with the composition, $\alpha_1\alpha_6\beta_{2/3}\gamma_{2S}\gamma_{2L}$ (Khan et al., 1994). Finally, several studies have shown that benzodiazepine pharmacology of the α_6 subunit is dominant over the α_1 subunit in receptor complexes that contain both subunits (Khan et al., 1994; Pollard et al., 1995); however, it should be noted that a recent study by Khan et al. (1996) has demonstrated that both the α_1 and α_6 subunits maintain their individual benzodiazepine properties. These data would suggest that α_1-directed benzodiazepine pharmacology would not be silent in these types of receptors.

In addition to the $\alpha_1\alpha_6\beta_{2/3}\gamma_2$ receptor complex, the $\alpha_6\beta_{2/3}\gamma_2$ complex has been reported to contribute 30–40% of total [^{3}H]Ro 15-4513 binding in the granule cell layer (Sieghart et al., 1987; Turner et al., 1991; Korpi et al., 1993; Quirk et al., 1994; Jones et al., 1997). Thus, about 20% of the benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding remains to be accounted for by a specific receptor subtype. Co-expression of δ with α_1/α_6 and a β has been shown to demonstrate benzodiazepine insensitivity (Shivers et al., 1989; Saxena and Macdonald, 1994, 1996). Apparently, benzodiazepine-insensitive $GABA_A$ receptors are a heterogeneous population. An α_6 knockout mouse made by homologous recombination showed not only an absence of benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding, but as well the α_6 subunit gene inactivation caused an inhibition of δ subunit expression (Jones et al., 1997). This study also indicated that α_1 does not combine with δ to form a benzodiazepine-insensitive $GABA_A$ receptor complex. Another observation that contributes to the knowledge of benzodiazepine-insensitive receptor subunit composition is that in general γ_2 and δ mRNAs are seen in separate neurons; and therefore, probably do not combine into the same receptor complex (Shivers et al., 1989). Moreover, Huh et al. (1996) have shown that γ_2 subunits are present in a significant fraction of benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding sites in the cerebellum. With respect to the work mentioned above, these data suggest therefore, that benzodiazepine-insensitive $GABA_A$ receptors are potentially composed of four subtypes; $\alpha_6\beta_x\delta$, $\alpha_6\beta_x\gamma_2$, $\alpha_1\alpha_6\beta_x\gamma_2$ and $\alpha_1\alpha_6\beta_x\delta$, where the β_x represents interchangeable β_2 or β_3 subunits. An immunoaffinity chromatography study by Jechlinger et al. (1998) has demonstrated that these subtypes represent, 14%, 32%, 37%, and 15%, respectively, of benzodiazepine-insensitive $GABA_A$ receptors in the cerebellum. With respect to the β subunit, it has been shown that furosemide is inactive in $\alpha_6\beta_1\gamma_2$ combinations, which confirms the notion of β_2 and/or β_3 being the natural in vivo β-subunit partner of the α_6 subunit (Korpi et al., 1995). The potential subunits and receptor subtypes repre-

Table 1
Benzodiazepine pharmacology of $GABA_A$ receptors in developing cerebellar granule cells

	Embryonic	Postnatal days 1–6	After postnatal day 6
Classic pharmacology[a]	Type $1/BZ_1$ and Type $2/BZ_2$	Type $1/BZ_1$	Type $1/BZ_1$
BZ-S/BZ-IS[b]	BZ-S	BZ-S	BZ-S and BZ-IS
Subunit mRNAs[c]	α_2, α_3, β_3, γ_1, γ_2, γ_3	α_1, β_2, β_3, γ_2	α_1, α_6, β_2, β_3, γ_2, δ
Pentameric complexes[d,e,f]	NR	BZ-S: $\alpha_1\beta_{2/3}\gamma_2$	BZ-S: $\alpha_1\beta_{2/3}\gamma_2$ $\alpha_1\alpha_6\beta_{2/3}\gamma_2$ BZ-IS: $\alpha_6\beta_{2/3}\gamma_2$ $\alpha_6\beta_{2/3}\delta$ $\alpha_1\alpha_6\beta_{2/3}\gamma_2$ $\alpha_1\alpha_6\beta_{2/3}\delta$

BZ-S: benzodiazepine-sensitive.
BZ-IS: benzodiazepine-insensitive.
NR: not resolved
[a] From Chisholm et al. (1983).
[b] From Korpi et al. (1993).
[c] From Laurie et al. (1992b).
[d] From Lüddens et al. (1990).
[e] From Jechlinger et al. (1998).
[f] From Khan et al. (1996).

senting different benzodiazepine pharmacology during cerebellar development are summarized in Table 1.

2.3. Electrophysiological aspects of $GABA_A$ receptors in the developing cerebellum

2.3.1. Pharmacology

The developmental changes in $GABA_A$ receptor subunit expression and benzodiazepine pharmacology taking place in the second postnatal week of the cerebellum also contribute to changes in receptor function. Primary cultures of granule cells have been an effective tool for studying the functional as well as biochemical changes which occur in the maturing granule cell layer. Mathews et al. (1994) have shown that granule cell sensitivity to benzodiazepines changes over time in culture. Potentiation of GABA-induced currents by benzodiazepines diminishes dramatically by the second week in culture, and this decrease in benzodiazepine-modulation of $GABA_A$ receptor function reflects a concomitant increase in α_6 mRNA in these cultures (Mathews et al., 1994; Zheng et al., 1994). Furthermore, the appearance of the α_6 subunit in primary cultures of granule cells is correlated with an increase in GABA potency in activating $GABA_A$ receptor-mediated Cl^- ion currents and an increase in susceptibility to current desensitization (Zheng et al., 1994). The maximal current induced by $GABA_A$ receptors, however, does not change over a culture period of 5–20 days in vitro (Zheng et al., 1994). When expressing $\alpha\beta\gamma$ recombinant receptors in HEK cells, the functional properties of $\alpha_1\beta_2\gamma_2$ and $\alpha_6\beta_2\gamma_2$ $GABA_A$ receptors emulated the electrophysiological responses recorded in early (5–7 days in vitro) and mature (14–20 days in vitro) primary granule cells, respectively (Mathews et al., 1994). Taken together, these results suggest that the $\alpha_6\beta_2\gamma_2$ $GABA_A$ receptor combination is highly sensitive to GABA and plays an important role in mediating benzodiazepine-insensitive GABA-induced currents in maturing granule cells.

Although the onset of benzodiazepine-insensitive pharmacology appears to be most closely related to onset of α_6 subunit expression (Zheng et al., 1994), the δ subunit could also be a contributor to the benzodiazepine-insensitive pharmacology seen in the granule cell layer. Studies of recombinant receptors have shown that the $\alpha_6\beta_2\delta$ combination can assemble into a benzodiazepine-insensitive $GABA_A$ receptor (Saxena and Macdonald, 1994). As with the α_6 subunit, the expression of the δ subunit also peaks in the second postnatal week (Laurie et al., 1992b), and this period in development also may be critical to the inhibitory effects of Zn^{2+} on $GABA_A$ receptor function. Saxena and Macdonald (1994, 1996) have demonstrated that $GABA_A$ receptor Cl^- ion currents are potently inhibited by Zn^{2+}, when the α_6 and/or the δ subunits are present. Further investigation is needed, however, in order to elucidate the effect of Zn^{2+} on developing granule cells. It also should be noted that the presence of the δ subunit in $GABA_A$ receptors prevents positive neurosteroid modulation of $GABA_A$ receptor function (Zhu et al., 1996). Zhu et al. (1996) noted that neurosteroid potentiation of GABA-gated Cl^- ion currents was present in cultured granule cells at 4 days in vitro but diminished by 14 days in vitro. These changes in the sensitivity of $GABA_A$ receptors in primary granule cells to neurosteroids were consistent with an increase in expression of δ subunit mRNA between 4 and 14 days in vitro (Zhu et al., 1996). In addition to the δ subunit conferring longer channel opening times, minimized desensitization rates, and increased GABA potency in $GABA_A$ receptors (Saxena and Macdonald, 1994, 1996), the developmental expression of the δ subunit in cerebellar granule cells may play an important role in the pharmacological modification (i.e.,

benzodiazepines, Zn^{2+}, and neurosteroids) of $GABA_A$-mediated, inhibitory synaptic potentials in the maturing cerebellum.

2.3.2. $GABA_A$ receptor Cl^- ion channel properties and kinetics

There have been few studies investigating the nature and kinetics of spontaneous inhibitory postsynaptic currents in the developing cerebellum. In granule cells, these spontaneous inhibitory postsynaptic currents appear to be primarily mediated by the activation of $GABA_A$ receptors (Wall and Usowicz, 1997). However, the nature and frequency of the spontaneous, $GABA_A$ receptor-mediated currents change during postnatal development. Voltage-clamp recordings from rat cerebellar slices have indicated that between postnatal days 7–14, spontaneous inhibitory postsynaptic currents occurred at a high frequency in more than half of the granule cells studied (Wall and Usowicz, 1997). With increasing age (i.e., postnatal days 18–53), the spontaneous inhibitory postsynaptic currents became less prevalent and an underlying tonic current became more prominent in granule cell recordings (Wall and Usowicz, 1997). The early spontaneous inhibitory postsynaptic currents could be blocked by bicuculline and tetrodotoxin, indicating an action potential-dependent phasic release of vesicular GABA. The bicuculline-sensitive tonic current could be blocked by tetrodotoxin in the mid-age group animals (i.e., postnatal days 18–25); however, tetrodotoxin sensitivity of the tonic current disappeared in the older animals (i.e., postnatal days 35–53). In adult animals, the tonic current was described to be an action potential-independent release of nonvesicular GABA or the neuromodulator, taurine (Wall and Usowicz, 1997). How the spontaneous inhibitory postsynaptic currents and bicuculline-sensitive tonic currents relate to $GABA_A$ receptor subunit composition remains to be fully elucidated.

The change in GABA-mediated synaptic transmission during development has also been noted by Brickley et al. (1996). Recordings from cerebellar slices from rats of different ages have shown that discrete, spontaneous postsynaptic currents decreased with age, being most prevalent at postnatal day 7, comprising 95% of the bicuculline-sensitive currents. Conversely, a tonic conductance mediated by GABA increased from 5% to 99% over the period from postnatal days 7–21 (Brickley et al., 1996). It should be noted that at postnatal day 7, bicuculline-sensitive spontaneous postsynaptic potentials were of a depolarizing nature, and GABA-activated potentials became totally inhibitory by postnatal day 18. Brickley et al. (1996) concluded that the bicuculline-sensitive tonic current appeared to reflect the activation of synaptic and extrasynaptic $GABA_A$ receptors. Due to increased complexity of the cerebellar glomerulus during development, GABA-activated synaptic and extrasynaptic receptors would be stimulated by an overspill of synaptically-released GABA and by a restricted diffusion of the neurotransmitter in the cerebellar glomerulus.

The decay kinetics of spontaneous inhibitory postsynaptic currents and GABA-activated currents also have demonstrated developmental changes in the cerebellum. When comparing the decay kinetics of spontaneous inhibitory postsynaptic currents from cerebellar slices prepared from postnatal days 7–30, spontaneous inhibitory postsynaptic currents recorded from younger granule cells (postnatal days 7–9) demonstrated slower decay kinetics than spontaneous inhibitory postsynaptic currents from slices older than postnatal day 14 (Tia et al., 1996b). Thus, these recordings revealed that the percentage of granule cells demonstrating fast decay kinetics increased with age. Consistent with Wall and Usowicz (1997), whole cell recordings from cerebellar slices also showed that the frequency of spontaneous inhibitory postsynaptic currents decreased with postnatal maturation (Tia et al., 1996b). In order to correlate the developmental changes observed in spontaneous inhibitory postsynaptic currents with functional properties of the $GABA_A$ receptor, GABA-activated currents were studied in outside-out nucleated patches from developing granule cells. In these recordings, the decay kinetics of spontaneous inhibitory postsynaptic currents and GABA currents matched better in younger granule cells (i.e., postnatal days 8–10) than from older granule cells (i.e., postnatal days 20–24), (Tia et al., 1996b). Similar to the spontaneous inhibitory postsynaptic current recordings, the slow decay kinetics of GABA-activated currents were more prominent in younger granule cells than older ones; however, GABA-activated currents from nucleated patches of older granule cells showed a lower percentage of fast decay kinetics than spontaneous inhibitory postsynaptic currents (Tia et al., 1996b). Since the nucleated patch recordings probably represent extrasynaptic $GABA_A$ receptors on granule cells, and the spontaneous inhibitory postsynaptic current recordings represent synaptic receptors, it was hypothesized that the $GABA_A$ receptor population at these two sites is similar early in development, but these populations of $GABA_A$ receptors become more distinct with age. This theory was supported by the finding that furosemide, a selective antagonist of $GABA_A$ receptors containing α_6 subunits (Korpi et al., 1995), did not inhibit spontaneous inhibitory postsynaptic currents until postnatal day 10, a time consistent with developmental expression of α_6 mRNA (Laurie et al., 1992b; Tia et al., 1996b). Furthermore, furosemide appeared to be ineffective in inhibiting GABA-activated currents from outside-out nucleated patches of granule cells at any time recorded during postnatal development (Tia et al., 1996b). These results would indicate that the α_6 subunit becomes potentially part of a $GABA_A$ receptor found at synaptic sites by postnatal day 10.

In trying to elucidate molecular determinants of spontaneous inhibitory postsynaptic currents and GABA-activated current kinetics found in developing granule cells,

GABA-activated currents have been studied in transfected mammalian cells expressing recombinant $GABA_A$ receptors of a defined subunit composition (Tia et al., 1996a,b). The co-expression of the α_6 subunit with β_2 or $\beta_2\gamma_2$ displayed slow deactivation kinetics and lacked desensitization in the presence of prolonged GABA applications (Tia et al., 1996a). Furthermore, the fast decay component of currents elicited by short GABA application was prominent in $\alpha_1\beta_2$, $\alpha_1\beta_2\gamma_2$ and $\alpha_1\alpha_6\beta_2\gamma_2$ receptor combinations. Taken together with the recordings from native receptors from younger granule cells (i.e., postnatal days 7–14), the relative contribution of fast decay kinetics in GABA-activated currents appeared to match best with the $\alpha_1\beta_2\gamma_2$ receptor combination. The predominance of the fast decay component in GABA-elicited currents in $\alpha_1\beta_2$ and $\alpha_1\alpha_6\beta_2\gamma_2$ receptors was comparable to recordings from granule cells in older rats (i.e., older than postnatal day 20). The $\alpha_6\beta_2\gamma_2$ combination did not match the kinetics of GABA-elicited currents recorded from native receptors of any age group. Although the fast decay component for some native $GABA_A$ receptors in developing granule cells could be matched with potential subunit compositions, there is still a discrepancy between the slow decay time constant of spontaneous inhibitory postsynaptic currents and GABA-elicited currents from native and recombinant $GABA_A$ receptors. Tia et al. (1996b) suggest that additional factors, such as receptor phosphorylation could play a role in determining spontaneous inhibitory postsynaptic current decay in early developing granule cells.

As discussed above, early developing granule cells (i.e., postnatal days 7–14) display primarily phasic $GABA_A$ receptor-mediated inhibition, as spontaneous inhibitory postsynaptic currents, with slow decay kinetics (Brickley et al., 1996; Tia et al., 1996b; Wall and Usowicz, 1997). The fast decay component in these early developmental currents appears to match the decay kinetics recorded from recombinant $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors (Tia et al., 1996a). Later in development the spontaneous inhibitory postsynaptic currents become less prominent yet display a higher percentage of fast decay kinetics. Moreover, a tonic bicuculline-sensitive current becomes the main inhibitory transmission recorded in granule cells, which appears to be the result of activated $GABA_A$ receptors at synaptic and extrasynaptic sites via a nonvesicular transmitter (Brickley et al., 1996; Tia et al., 1996b; Wall and Usowicz, 1997). What are the potential $GABA_A$ receptor subtypes involved in mediating these distinct types of inhibitory currents later in development? Furosemide inhibition of spontaneous inhibitory postsynaptic currents in older granule cells and the correlation between the fast decay kinetics of the recombinant $\alpha_1\alpha_6\beta_2\gamma_2$ receptor and native granule cell currents taken from older animals would indicate that this receptor combination potentially mediates inhibitory current via GABAergic synapses on developing granule cells (Tia et al., 1996a,b). Furthermore, considering that tonic GABAergic current may result from nondesensitizing $GABA_A$ receptors, a receptor subtype containing either the α_6 and/or the δ subunit may potentially display this type of persistent channel opening found in the maturing granule cell layer. Nevertheless, additional work is needed to elucidate the molecular determinants of all phasic and tonic $GABA_A$ receptor-mediated currents in the developing cerebellum, but it already seems evident that distinct $GABA_A$ receptor populations are involved in controlling granule cell output during cerebellar maturation.

3. Neurodifferentiating factors in cerebellar $GABA_A$ receptor development

The regulatory factors for the developmental expression of $GABA_A$ receptor subunits also have been reviewed by Wisden et al. (1996). Since its publication, there have been additional findings, albeit primarily from granule cells, which have brought more insight and questions to the complexity of $GABA_A$ receptor expression in the developing cerebellum. Elucidating the mechanisms regulating $GABA_A$ receptor subunit expression is important for understanding how $GABA_A$ receptor pharmacology evolves into its adult form in the cerebellum.

3.1. Extrinsic regulation

The factors regulating $GABA_A$ receptor subunit expression (summarized in Table 2) have been most extensively studied in cerebellar granule cells, be it in situ or in vitro. The rodent granule cell layer (the mammalian model most used) is established within the first 3 weeks of postnatal development (see Section 1), and it appears it is during this critical time of maturation and synaptogenesis that environmental cues will have their most potent effect on $GABA_A$ receptor differentiation. Typically, mRNAs encoding for $GABA_A$ receptor subunits, such as α_1, α_6, β_2 and γ_2, are low in the first postnatal week but then rapidly increase in the second postnatal week (Gambarana et al., 1990; Zheng et al., 1993). This increase in mRNA expression appears to be developmentally-regulated, because granule cells cultured at embryonic day 19 demonstrated constant mRNA levels for the α_1, β_2 and γ_2 subunits over a 21 day culture period; however, granule cells cultured on postnatal day 10 showed increases comparable to those found in vivo for the β_2 and γ_2 subunits (Beattie and Siegel, 1993). These results indicate that granule cells encounter a developmental signal by postnatal day 10, which elicits an increase in mRNA expression of $GABA_A$ receptor subunits. Furthermore, when monitoring the mRNA expression of granule cells cultured at 2 day intervals from postnatal days 2–10, Behringer et al. (1996) observed that the mRNA levels for both α_1 and α_6 subunits did not change over the culture period at all ages tested. The expression of β_2, β_3 and γ_2 subunit gene

Table 2
Summary of environmental cues regulating $GABA_A$ receptor subunit expression at the mRNA and/or protein levels during postnatal development

Subunits	Depolarizing medium-K^+	Excitatory neurotransmission (e.g., NMDA, glutamate)	Ca^{2+}/calmodulin-dependent protein kinases	cAMP, forskolin	$GABA_A$ neurotransmission (e.g., GABA, THIP)
α_1	↑[d]; NC[c]	↑[d]; NC[g]	ND	↑[f]	↑[b,e]
α_6	NC[c,d]	NC[d,g]	ND	↓[f]	↑[a]
β_1	ND	ND	ND	ND	NC[e]
$\beta_{2/3}$	NC[c]	ND	ND	ND	↑[b,e]
γ_2	NC[c]	ND	ND	ND	NC[e]
δ	↑[c]	ND	↑[c]	ND	NC[e]

ND: no data.
NC: no change.
[a] From Carlson et al. (1997).
[b] From Elster et al. (1995).
[c] From Gault and Siegel (1997).
[d] From Harris et al. (1995).
[e] From Kim et al. (1993).
[f] From Thompson et al. (1996a).
[g] From Thompson et al. (1996b).

transcripts were age-dependent; mRNA upregulation was evident beginning on postnatal day 8 (Behringer et al., 1996). The δ subunit mRNA increased in culture regardless of age; therefore, it was found to be dependent on cell density (Behringer et al., 1996). Taken together, it appears that the α subunits require a more constant signal not provided from the culture medium; whereas the β's and γ_2 subunits receive an environmental cue between postnatal days 6 and 8, which is sufficient to cause an increase in mRNA product. Moreover, cell–cell contact may play an important role for δ subunit mRNA expression (Beattie and Siegel, 1993; Behringer et al., 1996). These results suggest that the expression of the $GABA_A$ receptor subunit families is differentially regulated during postnatal development.

Considering that these developmental changes in $GABA_A$ receptor subunit expression are coincidental with a period of synaptogenesis in the granule cell layer, excitatory contacts from mossy fibers and/or inhibitory contacts from Golgi neurons are likely candidates for regulating $GABA_A$ receptor subunit expression. Depolarizing medium has been shown to upregulate the α_1 and δ subunits at the transcriptional level, but not the α_6, β_2 and γ_2 subunits (Harris et al., 1995; Gault and Siegel, 1997). Furthermore, second messenger systems may be implicated in the regulatory effects of neuronal activity. As for the δ subunit, transcriptional expression was reduced in the presence of Ca^{2+}/calmodulin-dependent protein kinase inhibitors (Gault and Siegel, 1997). In this experiment, granule cell cultures were exposed to these protein kinase inhibitors for 2 days in vitro. Thus, calmodulin-kinases appear to play an integral role in modulating δ subunit mRNA transcription directly or the events that lead to its transcription. Thompson et al. (1996a) have shown that chronic exposure (i.e., 7 days in vitro) to agents that enhance cAMP concentrations, such as forskolin, increased α_1 protein expression as well as benzodiazepine-sensitive [^{3}H]Ro 15-4513 binding. On the contrary, α_6 protein levels were decreased in the presence of forskolin. Whether cAMP control of $GABA_A$ receptor subunit expression is mediated at the transcriptional, translational, and/or receptor level is a question which remains to be elucidated. Finally, there is conflicting evidence with regard to excitatory regulation of the postnatal expression of $GABA_A$ receptor subunits via ionotropic glutamate receptors. It has been demonstrated that the α_1 subunit transcriptional rate can be significantly enhanced in response to acute NMDA treatment (Harris et al., 1995). Also in cultured granule cells, benzodiazepine potentiation of $GABA_A$ receptor responses, which indirectly suggests an effect on the α_1 and γ_2 subunits, is also augmented with acute exposure to NMDA (Zhu et al., 1995). Thompson et al. (1996b) have observed that chronic exposure (i.e., 9 days in vitro) to antagonists of non-NMDA and NMDA receptors do not alter benzodiazepine-sensitive and benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding or α_6 protein levels in primary cultures of granule cells. The authors from the latter study do not exclude the possibility that excitatory regulation of α_1 and α_6 subunit expression during postnatal development may occur via the metabotropic glutamate receptor family. Nevertheless, it is important to note that a depolarization signal which is mediated by excitatory amino acid receptors and received by granule cells early in postnatal development plays a permissive role in granule cell differentiation and survival (Balazs et al., 1988, 1990).

GABAergic transmission, namely from $GABA_A$ receptors, has also been shown to act as a neurodifferentiating factor in early cerebellar development (Hansen et al., 1987; Wolff et al., 1987). It still remains controversial whether GABAergic transmission is a depolarizing or hyperpolarizing current early in development, yet GABA and $GABA_A$ receptor agonists, such as THIP, can promote the

appearance of low affinity GABA binding and the de novo synthesis of its own receptor subunits in primary cultures of granule cells (Meier et al., 1984; Kim et al., 1993; Elster et al., 1995; Carlson et al., 1997). The $GABA_B$ receptor agonist, baclofen, has been shown to have no effect on promoting low affinity GABA binding (Meier et al., 1984). Even after an acute exposure (i.e., 6 hours) to GABA or THIP, an increase in the gene transcripts for the α_1 and β_2 subunits, which can be blocked by the $GABA_A$ antagonist, bicuculline, has been observed in cultured granule cells (Kim et al., 1993). The β_1, β_3, γ_2 and δ subunit mRNA levels appear not to be affected by this acute exposure to $GABA_A$ receptor agonists. Using electron microscopy, Elster et al. (1995) have demonstrated that exposure to THIP for 48 hours induced an increase in the immunogold labeling of the α_1 and $\beta_{2/3}$ subunits in primary cultures of granule cells. This increase was displayed in both the cell bodies and processes of granule cells. After an additional exposure to THIP for 96 hours, only the granule cell processes expressed an upregulation of α_1 and β_2 protein (Elster et al., 1995). Likewise, the neurodifferentiative effect of THIP has been observed at the mRNA and protein levels for the α_6 subunit (Carlson et al., 1997). In this study after 48 hours of exposure, THIP enhanced photolabeled benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding to a 56 kDa peptide, (i.e., the α_6 subunit); α_6 subunit clustering; and α_6 subunit mRNA expression in cultured granule cells (Carlson et al., 1997). After an additional 96 hours of exposure to THIP, α_6 mRNA and protein levels were shown not to be different from control cultures. These findings are in agreement with a study demonstrating that α_6 subunit protein and benzodiazepine-insensitive [^{3}H]Ro 15-4513 binding increases in primary cultures of granule cells from 3–7 days in vitro, but then plateaus after 7 days in vitro (Thompson and Stephenson, 1994). Moreover, the finding that photolabeled [^{3}H]Ro 15-4513 binding to a 51 kDa protein (i.e., the α_1 subunit) increased after the initial exposure to THIP but displayed a decrease after further exposure to the $GABA_A$ receptor agonist (Carlson et al., 1997). This result indicated that the α_1 subunit as compared to the α_6 subunit may be regulated differentially by THIP (Carlson et al., 1997). The photolabeled [^{3}H]Ro 15-4513 binding to a 51 kDa protein in the study by Carlson et al. (1997) is also consistent with the observations from Thompson and Stephenson (1994), that α_1 protein continued to increase in cerebellar granule cell culture from 1–9 days in vitro; however, the benzodiazepine-sensitive [^{3}H]Ro 15-4513 binding decreased from 5–7 days in vitro. It is important to note that in the studies of both Carlson et al. (1997) and Thompson and Stephenson (1994) granule cells were cultured from postnatal day 7 cerebella. These results suggest that not all of the α_1 subunit protein being synthesized during later postnatal development is incorporated into functional benzodiazepine-sensitive binding sites on $GABA_A$ receptors. Furthermore, the enhancing effect of THIP on α_6 subunit expression is limited to an early developmental stage rather than extending throughout the entire culture period (Carlson et al., 1997). An earlier study has shown that THIP does not have a neurotrophic effect on either Type 1 or Type 2 benzodiazepine binding sites on granule cells at any time during the culture period of 4–15 days in vitro (Squires et al., 1990). Thus, it could be suggested that THIP-induced regulation of the expression of $GABA_A$ receptor subunits may be selective to subunits comprising benzodiazepine-insensitive receptors. Finally, auto-regulation of $GABA_A$ receptor expression is not exclusive to the developing cerebellum, as it has been observed in cultures derived from the cerebral cortex, as well (Poulter et al., 1997).

3.2. Intrinsic regulation

Not much is known about the intrinsic clock which may be partially responsible for the changes in $GABA_A$ receptor subunit expression during cerebellar development. From in situ hybridization, it has been shown that the early postnatal cerebellum expresses a set array of $GABA_A$ receptor subunits. Purkinje cells express α_1, β_2, β_3 and γ_2 mRNAs; Bergmann glia contain mRNA for the α_2 and γ_1 subunits; and granule cells display the α_2, α_3, some α_1, β_1, β_3, γ_1, γ_2 and γ_3 gene transcripts (Laurie et al., 1992b). The subunit mRNAs for stellate/basket cells, however, could not be resolved during early postnatal development, but in the adult cerebellum, these cells express mRNA for the α_1, β_2, and γ_2 subunits (Laurie et al., 1992a). Although the expression of $GABA_A$ receptor subunit mRNAs in Purkinje cells remains constant, a developmental switch occurs postnatally for the granule cell layer. In this case, the α_1, α_6, β_2, β_3, γ_2, δ mRNAs dramatically increase and the α_2, α_3, β_1, γ_1, γ_3 transcripts decrease (Laurie et al., 1992b). As discussed above, environmental cues appear to be necessary for the upregulation of subunits in the granule cell layer during early development (refer to Table 2). However, it remains to be seen how the other subunits (i.e., α_2, α_3, β_1, γ_1, γ_3) are down-regulated during postnatal development. Perhaps, this decrease is controlled by an intrinsic timing mechanism on gene expression.

In summary, there is an orchestrated timing of intrinsic and extrinsic mechanisms regulating $GABA_A$ receptor subunit expression during granule cell development. The extrinsic factors can vary from neurotransmitters to second messengers; however, their modulatory properties are typically coincident with a period of neuronal maturation in vivo. Furthermore, it appears that the effects of these extrinsic factors are heterogeneous within and across $GABA_A$ receptor subunit families. Due to the fact that $GABA_A$ receptor subtypes are differentially located across the granule cell membrane (Nusser et al., 1998), and that these subtypes are pharmacologically and most likely functionally distinct, it seems reasonable that the expression of

the $GABA_A$ receptor subunit families would be regulated differentially.

4. Concluding remarks

From the work reviewed above, it appears that the developmental changes in the pharmacological and hence the molecular subtypes of $GABA_A$ receptors can influence the granule cell layer's communication to its cerebellar targets. A recent study by Zhu and Vicini (1997) has shown that neurosteroids prolonged the decay kinetics of GABA-gated currents; however, this increase in the slow decay time constant was more prominent early in granule cell development (i.e., postnatal days 10–13) as opposed to later (i.e., > postnatal day 30). These data would suggest that endogenous modulators, such as neurosteroids, would differentially regulate GABAergic transmission during development, and that this differential regulation would be dependent on the developmental expression of $GABA_A$ receptor subtypes, containing a δ subunit (Zhu and Vicini, 1997). How important is one subunit in the cerebellum to development at the whole animal level? This question has been approached by using gene specific knock-out mice models. It appears at least at the behavioral level, when monitoring cerebellar-associated motor skills, α_6 knock-out mice, which also exhibit a depletion in the δ subunit, are comparable to their wildtype littermates (Jones et al., 1997). However, a gene knock-out of a more ubiquitous subunit, such as the γ_2, hindered the postnatal growth, sensorimotor and behavioral functions of mutant mice, albeit embryonic development appeared unaffected (Günther et al., 1995). If individual subunits can be critical to proper GABA-mediated inhibitory transmission in the developing cerebellum, then the factors regulating their expression will also play important roles in granule cell output. As mentioned above, GABA can act as an environmental cue for autoregulating its own receptor subunits. The intracellular mechanism for this neurodifferentiating effect has not been elucidated, but it appears that polyamines are a critical component. Abraham et al. (1994) have shown that THIP-induced expression of low-affinity $GABA_A$ receptors is negated in the presence of polyamine biosynthesis inhibitors. Considering that polyamines are found in proliferating cells and help stabilize double-stranded DNA in these cells, it could be suggested that the role of polyamines in the differentiative action of THIP may be to act as a permissive signal for de novo protein synthesis. Furthermore, proliferating and differentiating granule cells from rat cerebella have approximately a two week window of opportunity for regulation by extrinsic factors. Whether there is one window for all regulatory factors or various windows for each cue, it is currently unknown. These inquiries, as well as how long the window stays open, have yet to be determined.

To this end, the cerebellum as compared to other areas of the brain may display a more limited repertoire of $GABA_A$ receptor subunits, but it has been suggested from immunogold labeling that several $GABA_A$ receptor subtypes can colocalize at both synaptic and extrasynaptic sites on cerebellar granule cells (Nusser et al., 1998). This diversity leaves an extensive capacity for heterogeneous GABA-mediated inhibition in the neuronal circuitry of the granule cell layer. Thus, the process of postnatal $GABA_A$ receptor development allows for the proper fine-tuning of the granule cell output found in the adult cerebellum.

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