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Selective vulnerability of cerebellar granule neuroblasts and their progeny to drugs with abuse liability

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Abstract

Cerebellar development is shaped by the interplay of genetic and numerous environmental factors. Recent evidence suggests that cerebellar maturation is acutely sensitive to drugs with abuse liability including alcohol, opioids, and nicotine. Assuming substance abuse disrupts cerebellar maturation, a central question is to what are the basic mechanisms underlying potential drug-induced developmental defects. Evidence reviewed herein suggests that the maturation of granule neurons and their progeny are intrinsically affected by several classes of substances with abuse liability. Although drug abuse is also likely to target directly other cerebellar neuron and glial types, such as Purkinje cells and Bergmann glia, findings in isolated granule neurons suggest that they are often the principle target for drug actions. Developmental events that are selectively disrupted by drug abuse in granule neurons and/or their neuroblast precursors include proliferation, migration, differentiation (including neurite elaboration and synapse formation), and programmed cell death. Moreover, different classes of drugs act through distinct molecular mechanisms thereby disrupting unique aspects of development. For example, drug-induced perturbations in (i) neurotransmitter biogenesis, (ii) ligand and ion-gated receptor function and their coupling to intracellular effectors, (iii) neurotrophic factor biogenesis and signaling, and (iv) intercellular adhesion are all likely to have significant effects in shaping developmental outcome. In addition to identifying therapeutic strategies for drug abuse intervention, understanding the

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mechanisms by which drugs affect cellular maturation is likely to provide a better understanding of the neurochemical events that normally shape central nervous system development.

Keywords

neuroblast proliferation; *cerebellar development*; *programmed cell death*; *nicotinic acetylcholinergic receptors*; *opioid receptors*; *heroin*; *nicotine*

Introduction

A variety of classes of drugs with abuse liability affect cerebellar structure and function in adults.[1] Recent evidence suggests that many of these drugs can have profound affects on cerebellar development. This includes opiates, nicotine, and alcohol, but may additionally include stimulants such as cocaine and methamphetamine, as well as the non-equilibrium *N*-methyl-D-aspartate (NMDA) antagonist phencyclidine (PCP),[1] which are known to effect cerebellar function in adults. Neurotransmitter systems regulate many aspects of normal development. Although the mechanisms by which individual drugs of abuse affect neural maturation are not fully understood, it is assumed that many drugs act by mimicking or interfering with normal endogenous neurotransmitter-receptor interactions during maturation. The inference is that drug abuse alters neurodevelopment by disrupting the timing and sequence of developmental actions regulated by endogenous neurotransmitter systems. In this review, we focus on opioids and nicotine, and to a lesser extent alcohol (which has been more extensively reviewed elsewhere). Alcohol, opioids, and nicotine are reported to effect directly the maturation of granule cell precursors and their progeny. Stimulants such as cocaine or methamphetamine are more likely to affect neurochemical systems (e.g., norepinephrine and dopamine reuptake) that directly influence Purkinje cell maturation. Understanding how cerebellar development is perturbed will provide increasingly rationale approaches toward intervention in drug-exposed offspring, and should additionally provide insight into how neurochemical systems normally influence brain development.

The cerebellum is an important model system for understanding CNS development. The cerebellum is highly compartmentalized and the extremely ordered and stereotypic cytoarchitecture results from a tightly orchestrated production of neurons and glia during development.[2,2-16] Cerebellar development proceeds with such precision that it is a useful model for identifying perturbations in CNS maturation.

A hallmark of cerebellar development is the enormous production of granule cells or neurons, which outnumber neurons in other brain regions.[17] Granule neurons are relatively small in comparison to other neurons, with three-to-seven dendrites. Their axon runs vertically from the granule layer into the molecular layer, where it bifurcates as a parallel fiber and forms contacts with hundreds of Purkinje cells [13]. The huge increase in the number of granule neurons during development arises from the proliferative expansion of neuroblast precursors, which occurs in several steps. Initially, neuroblast precursors originating from the rhombic lips proliferate and migrate tangentially along the surface of the incipient cerebellum to form the external granular (or germinal) layer (EGL).[2,18-21] Cells within the

rhombic lip express *math1* and are largely committed to a granule neuron fate before they migrate to the EGL.[22-24] Second, EGL neuroblasts undergo a sustained period of proliferation. Recent evidence suggests that the EGL exclusively gives rise to granule neuron progenitors, rather than other molecular layer interneurons (stellate and basket cells) as previously thought.[25] Finally, postmitotic granule neurons migrate radially through the cerebellum, bypassing outwardly migrating Purkinje cells and settling in the internal granule layer (IGL).[26,27] The EGL disappears when neurogenesis is complete.[2,3]

The period of granule neurogenesis coincides with critical periods of sensitivity to several drugs of abuse. This corresponds approximately to the first three postnatal weeks in rats and mice [4,12], and the third trimester of gestation until 1.5 years in humans.[14] Superimposed on the rapid period of proliferation are significant amounts of programmed cell death.[28] The intense rate of granule neurogenesis is tightly regulated and coordinated with the maturation of the entire organism. The net production of granule cells is determined by proliferation and cell death and is modified by a variety of external cues (Fig. 1). Protracted neurogenesis, combined with a high degree of sensitivity to extrinsic factors, makes developing granule cells especially vulnerable to drug abuse.

Besides granule neurons, Purkinje neurons are a potential target for many abused substances during development (Table 1). Purkinje cells are generated before granule neurons and undergo a prolonged period of differentiation that is in part dependent on trophic support provided by granule neuron afferent synapses.[13,18,29-31] The profound trophic interdependence between Purkinje and granule neurons presents challenges toward sorting causal developmental relationships.[32] Despite this challenge, with recent cellular and genetic approaches, it is possible to identify key intercellular events influencing the development of each cell type. For this reason and others, we have made extensive use of *in vitro* methods to study how drugs intrinsically affect the maturation of granule cell precursors. However, the inherent trade-off for gaining experimental control *in vitro* is the loss of relevance resulting from a reductionist approach. A partial compromise has been to use organotypic culture, which retains some of the cell-to-cell interactions and three-dimensional organization inherent in the cerebellum.[33,34] Nevertheless, it is important to validate *in vitro* findings *in vivo*.

Alcohol

Alcohol has profound effects on the development of cerebellar granule neurons and their progeny. Cerebellar actions have been extensively studied as part of the global effects seen with fetal alcohol syndrome (FAS) and only briefly discussed here. One only has to attempt to “walk a straight line” following excess consumption to understand ataxia and appreciate the preferential effects of alcohol on cerebellar function as an adult. The effects of alcohol on the developing cerebellum are likely to be even more profound when alterations in neural function appear to contribute to lasting changes in the cytoarchitecture and synaptic circuitry. Alcohol perturbs all aspects of granule cell development; but is especially damaging to postmitotic cells, altering events such as neuronal migration and survival.

Studies by Li et al. demonstrate that the kinetics of the cell cycle is disrupted by exposure to ethanol. Alcohol downregulates the expression of cdk2, cyclin A and cyclin D causing a delay in the cell cycle and promoting apoptosis, which leads to an overall decrease in the cell number of granule neurons.[35] Faulty migration with ectopic positioning of cerebral cortical as well as other neuronal populations seems to be a major consequence of FAS.[36,37] How migration is affected is uncertain, but may relate to ethanol-induced disruptions in intracellular Ca^{2+} signaling,[38,39] or cell adhesion,[40,41] either of which might be important for granule cell migration.[42,43] Alcohol has been shown to modulate the activity of cell adhesion molecules important in cell-cell and cell-matrix interactions. Physiological concentrations of ethanol impair and in some cases inhibit the function of L1, a cell adhesion molecule responsible for mediating neurite outgrowth and perhaps the migration of granule cells.[40,41,44]

Another important target is granule neuron survival, which is modulated by ethanol *in vitro* and *in vivo*.[45-51] Alcohol exposure and withdrawal disrupts the function of NMDA[38,45,52-54], AMPA/kainate[55,56], and GABA [57-59] receptor-effector coupling,[53,55-57,60-63] as well as voltage-dependent Ca^{2+} channel function.[57] Ligand- and voltage-dependent channels are important regulators of neuroontogeny and survival.[38,64] In addition to ion homeostasis and mitochondrial function,[65] ethanol disrupts trophic factor biogenesis and neuronal responsiveness to trophic support. Pituitary adenylate cyclase-activating polypeptide[66], insulin-like growth factor[67,68], brain-derived neurotrophic factor [69-72], nerve growth factor and basic fibroblastic growth factor[73] all attenuate ethanol-induced granule neuron death. Because ethanol seemingly influences all aspects of granule neuron maturation, suggests that the mechanisms by which alcohol acts are complex and likely affect multiple systems. The ability of ethanol to perturb ion homeostasis, neurotransmitter or trophic factor biogenesis and/or receptor-effector coupling,[52,74-80], cell adhesion, as well as glial development and function[81,82] (however see [83,84]), are all likely to profoundly impact granule neurogenesis. As noted, excellent articles and reviews on alcohol and cerebellar development exist (Table 1).[46,57,76,85,86]

Opioids

The involvement of opioids in cerebellar growth regulation has been revealed by experimentally perturbing the endogenous opioid system.[33,34,87-93] In this review, “opiate” refers to substances that are derived from the opium poppy such as heroin or morphine, while “opioid” refers to endogenously expressed neuropeptides and receptors.[94] Heroin’s action in the CNS results in large part from its conversion to morphine. Endogenous opioid peptides and receptors are widely expressed by developing cerebellar cells.[95-103] Although heroin and morphine preferentially activate μ opioid receptors, at high concentrations they can activate δ and κ receptors.[104] Continuous opioid receptor blockade accelerates cerebellar growth in postnatal rats,[88,93,105,106] while over-stimulating opioid receptors , as occurs with opiate drugs,[107-112] retards cerebellar growth [review [88,91,93,105,106,113]]. This suggests that endogenous opioids are present during cerebellar development and tonically inhibit growth.

In the cerebellum, acute opioid exposure (≤ 72 h) typically inhibits the proliferation of cerebellar neuroblasts and astroglia,[88,93,105,105,106,109,114-119] and can affect cell

differentiation[33,88,105] and survival.[33] Opioid actions are complex and affect each cerebellar cell type differently.[120] For example, unlike neuroblasts and astroglia in which morphine inhibits cell replication, morphine is mitogenic to immature oligodendrocytes.[121] In another example, cell death is not seen with high concentrations of morphine (>1 μM) in cultured mouse granule cells[33,92] or astrocytes (unpublished, see also [33,122,123]), but cell death is evident in cultured Purkinje cells with more chronic exposure (~7 days).[33] The mechanism underlying Purkinje cell death is uncertain, but may result from morphine-induced reductions in parallel fiber afferents from granule cells.[33] Purkinje cell losses have been reported in chronic heroin abusers who are HIV-seronegative.[124]

Toxic heroin leukoencephalopathy

Recently a heroin induced spongiform leukoencephalopathy has been described that effects predominately the posterior fossa structures including the cerebellum.[125,126] Ultrastructural studies show vacuolar changes in the myelin. Recent identification of this entity, most likely reflects the increased popularity of the practice of "dragon chasing".[127] In this mode of heroin abuse, powdered heroin is placed on a piece of aluminum foil and heated from below with a flame. The oil content allows the heroin to liquefy and vaporize, producing a plume that is inhaled through the mouth with a straw or aluminum foil tube. This practice is distinct from smoking or sniffing heroin. Heroin chasers tend to be younger than heroin injectors, and this route of administration seems to appeal to users trying to avoid intravenous heroin use.[128] As drug users explore modes of administration that avoid the risk of HIV exposure, they may resort to heroin inhalation. This condition has never been reported in persons using heroin by other means, such as injection or snorting, which suggests that the extreme toxicity arises from the formation of one or more toxic byproducts during heroin volatilization or from the unusual pharmacodynamics of heroin exposure through this unique route of administration. The toxin in heroin-induced leukoencephalopathy is unknown, but progression in this condition might be due to coasting, or, alternatively, to persistent metabolic changes in the affected white matter such as ongoing oxidative damage initiated by a toxin. The illness is extremely grave, with no known treatment and progression to akinetic mutism and death in approximately 20% of reported cases.[129]

Opioid receptor and peptide expression in the cerebellum

There is considerable discrepancy between opioid peptide and receptor expression in immature and adult granule cells.[92,95-98,130,131] Immature cells within the EGL display proenkephalin mRNA and/or peptide products, which are lost with maturation.[96,97,130] Ontogenetic changes in proenkephalin expression within individual cells are manifest as dynamic spatiotemporal gradients in opioid neuropeptide levels throughout the entire cerebellum.[96]

Interestingly, high-levels of opioid receptor binding coincide with the transient appearance of the EGL.[98,130,132] Immature EGL cells from postnatal mouse cerebellum display immunocytochemical and functional evidence of μ and δ, but not κ, opioid receptor expression *in vitro*.[92] EGL cells express a putative ζ opioid receptor with high affinity for Met-enkephalin.[98,131] Initial reports identifying opioid receptors in the immature cerebellum were viewed with some skepticism, because the adult cerebellum in rats and mice has been

traditionally described as being largely devoid of opioid receptors. More recently, low levels of δ receptor expression have been reported in mature granule cells in rodents,[133,134] while μ receptors are reported absent. In contrast, in the human cerebellum, high affinity opioid binding sites are associated with the EGL[130] and μ opioid receptors are widely expressed in adult granule cells,[135,136] indicating species differences in the types opioid receptors present. The transient and coordinated expression of the opioid peptides and receptors in the developing cerebellum infers that they are functionally related to growth, and suggests granule cells are important in opioid-dependent maturation in the cerebellum.

Opioids and granule cell precursor maturation.

Preliminary evidence implicating endogenous opioids in neural development came from findings that heroin, morphine (much of heroin's actions in the CNS result from its conversion to morphine), or other preferential μ opioid receptor agonists inhibited the growth of the brain including the cerebellum.[118,137-139,139] We tested whether opiates intrinsically affect the growth of granule cell precursors by studying the response of mouse precursors to morphine *in vitro*. Morphine (1 μM) exposure caused significant reductions in DNA synthesis at 24 h with subsequent reductions in DNA content at 48 h.[92] Importantly, because morphine does not increase EGL cell death, suggests that morphine reduces granule neuron numbers by inhibiting neuroblast proliferation. Lastly, the antiproliferative effects of morphine appear to be mediated by μ opioid receptors, since granule cell precursor proliferation was unaffected by δ opioid receptor agonists and κ receptors are not expressed by these cells (Fig. 2).[92]

Opioids can modulate dendritic growth and/or potentially retraction.[34,88,92] In immature cerebellar granule cells, δ2 receptor agonists, but not μ receptor agonists, preferentially inhibit neurite elaboration. Proenkephalin gene-derived products have been noted in mossy fibers in a variety of species.[140,141] In another model system, Met-enkephalin, acting through δ receptors, significantly increases the phosphorylation of the Src kinase substrate cortactin and vinculin at focal adhesion sites,[112] suggesting one possible mechanism for opioid-induced changes in neurites. Prenatal exposure to morphine alters catecholamine levels in the cerebellum via a sexually dimorphic mechanism and might affect other neurotransmitters.[142]

Opioids act through multiple pathways and downstream effectors, including MAP kinase and/or focal adhesion kinase.[92,112,143-148] Opioids can also affect cell growth through pathways more traditionally ascribed to opioids, such as by augmenting phosphatidylinositol (PI) turnover, or by increasing PI-3-kinase and/or Ca^{2+}-mobilization.[145,149-152] The ability to stimulate multiple signaling cascades may explain how opioids can have varied effects on cell growth.[120,121,153,154]

Little is known whether opiates modulate development by altering key trophic factors and/or their receptors. We have found that heparin-binding epidermal growth factor (Hb-EGF) negates the antiproliferative actions of morphine in isolated mouse EGL cells.[155] Conversely, opiates can modify EGFR function through convergent signaling events.[148] Similarly, different classes of opioid receptors can transactivate one another[156-158] and interact directly with important non-opioid signaling pathways affecting growth.[159]

Interactions between opiates and other trophic regulators, such as sonic hedgehog (Shh)-patched2 and/or EGF-erbB receptor interactions between Purkinje and granule neurons have not been fully explored in the developing cerebellum. Assuming Shh-patched2 and/or EGF-erbB receptor interactions drive the near-exponential increases in granule neurogenesis, how might opioids interact with these potent trophic factors? We propose that opioids are strategically positioned to finely tune and coordinate developmental details, and are likely to function at later stages during development, after trophic factors such as EGF or hedgehog have served their main functions. In addition, as noted, opioid and growth factor signaling pathways can overlap intracellularly. Because opioid peptides and receptors are widely expressed by developing neurons, astroglia, and oligodendroglia suggest that opioids are strategically positioned to coordinate the proliferation and differentiation of neurons and glia.[92,160,161] This might include the regulation of neuronal and glial numbers, or physical or functional interactions among cells.[120] Irrespective of a particular mechanism, current evidence suggests that opioids affect cerebellar maturation by interfering directly with granule cell development.

Nicotine

AChR and transmitter expression in the developing cerebellum

The abundance of cholinergic synthetic enzymes and receptors in the developing cerebellum suggests that acetylcholine might potentially influence postnatal maturation in this region.[162-165] The maturation of cholinergic systems[164,166-168] coincides with critical periods of granule neurogenesis in rodents.[4,12,169] During this time, choline acetyltransferase levels are generally higher than levels of the degradative enzyme for acetylcholine, acetylcholinesterase.[163,164,170] Granule neurons receive cholinergic mossy fiber innervation from dorsal pontine brainstem nuclei late during development,[168] suggesting that acetylcholine affects synaptogenesis and neuromodulation.[171] Choline itself, which is plentiful during development, may activate α7 nicotinic AChRs and act as a partial agonist for α3 nicotinic AChRs.[172-174]

Both muscarinic and nicotinic AChR subtypes are present in perinatal rat[175-177] and human brains.[167,178] Nicotinic AChRs are expressed in the EGL in humans[167] and in granule and/or Purkinje neurons in rodents[165,175,179,180] and can precede the ingrowth of cholinergic axons,[181] suggesting that nicotinic agonists could act directly on granule neuron precursors. Our immunocytochemical findings show α3, but not α4, nicotinic AChRs in cultured EGL cells prior to the formation of neurites.[180] Interestingly, transcripts of multiple nicotinic AChR subtypes, including α3, α4, α5, α7, β2, and β4, have been detected in more mature cultured rat granule neurons that have formed axons and dendrites.[182] Similarly, α4β2 and/or α3β4 nicotinic AChR subunits are expressed by granule neurons in cerebellar slices from 5 to 14 day-old rats,[183] suggesting that nicotinic AChR subunit composition is developmentally regulated and that non-α3 subtypes might be more important for neurite outgrowth and synapse formation. Additional support for this is prompted by the finding that α7 subunits are segregated to the dendrites, but not cell bodies of granule cells, suggesting α7 subunits are preferentially involved in differentiation, synaptogenesis, and/or postsynaptic function.[184] In contrast, α3 nicotinic AChR subunits are localized on the cell

bodies of neuroblasts long-before neurites are formed.[180] In 5-to-10 day old rats, nicotine significantly enhances synaptic activity of the Purkinje cells via presynaptic nicotinic receptors on the excitatory and inhibitory interneurons, while in older rats such an effect is barely noticeable.[185]

Nicotine and granule cell precursor maturation

Together, these findings suggest that the cholinergic system is important in cerebellar maturation, at least in part, by directly influencing the proliferation and survival of granule cell precursors. Nicotine administration has been reported to decrease DNA synthesis in several rat brain regions including the cerebellum,[186-188] although a recent report shows increased numbers of mitotic neural cells in rat embryos exposed to nicotine *in vitro*.[189] However, similar to opioids, nicotinic AChR activation has potent systemic effects, which include alterations in cardiovascular, respiratory, and endocrine function that are likely to influence neurogenesis. Activation of presynaptic nicotinic AChRs might modulate the release of other neurotransmitters. Recently, abnormalities in nicotinic AChR subunit levels have been reported in autism.[190]

To understand better the intrinsic effects of nicotine on the development of granule cell precursors, we examined the effect of nicotine on EGL cells isolated *in vitro*. We found that nicotine caused concentration-dependent increases in DNA content and synthesis in EGL neuroblasts implying increases in cell proliferation. Pretreatment of cultures with the nicotinic AChR antagonist dihydro-β-erythroidine (DHBE) significantly attenuated nicotine-induced increases in cell replication. To further determine whether α3 or α4 subunits are preferentially involved in neural proliferation, EGL cultures were continuously exposed for 7 days to selective α3/α4 (epibatidine) or α4 (cytisine) agonists or partial agonists, and DNA content and synthesis were examined.[180] Epibatidine, but not cytisine, caused concentration-dependent increases in DNA synthesis and DNA levels in EGL cells indicating that α3 nicotinic AChR activation is mitogenic. Moreover, significant effects were seen with a 1 pM concentration of epibatidine, and were markedly attenuated by concurrent administration of DHBE suggesting the involvement of specific nicotinic AChRs. In summary, these data provide novel evidence that nicotinic AChRs directly affect the development of granule cell precursors and further suggest that the effects are mediated through α3 nicotinic AChR subtypes. It is interesting to speculate that other nicotinic AChR subtypes also regulate unique aspects of development.

Cell Death

Granule neuron death occurs during normal cerebellar development *in vivo*[28] and *in vitro*.[191] Nicotinic AChR activation can have paradoxical proapoptotic or neuroprotective effects depending on cell type and developmental stage, pharmacodynamics of drug exposure, and the particular nicotinic AChR subtype affected. Chronic nicotine exposure is neuroprotective in organotypic cultures of the hippocampus by upregulating calbindin expression, which buffers toxic increases in intracellular Ca^{2+}.[192] In hippocampal neurons, neurotoxicity is associated with the activation of α7 nicotinic AChR subtypes, which permit Ca^{2+} influx and α7 antagonists can be neuroprotective.[193,194] In contrast, there are numerous examples in which nicotine is neurotoxic. Nicotine promotes death in some cell types, e.g., embryonic rat

neural cells[189] and in vascular cells.[195] Nicotine is neurotoxic at high concentrations in whole rat embryos,[189] and causes apoptosis in cultured hippocampal neurons.[193]

We found that EGL cell viability was enhanced following chronic nicotine treatment for 7 days *in vitro* (DIV), but not 4 DIV.[180] Importantly, the neuroprotective effects of nicotine were completely blocked by the nicotinic AChR antagonist DHBE and mimicked by α3, but not α4, selective agonists. Chronic exposure may be neuroprotective by causing adaptive responses within cells.[192] Alternatively, the background rate of cell death was greater in our 7 DIV cultures and this might better reveal nicotine neuroprotection.

Collectively, the cell proliferation and survival data suggest that nicotine has both mitogenic and neuroprotective effects in EGL cells, and these effects are mediated through α3 nicotinic AChR subunits (Fig. 2). Interestingly, Yan and coworkers[196] reported that acetylcholine prevented apoptosis in cultured granule neurons via an interaction with muscarinic AChRs. It is conceivable that activation of both nicotinic AChR and muscarinic AChRs regulate the maturation of cerebellar granule neurons as has been suggested in retinal ganglion cells.[197] Alternatively, nicotinic AChR stimulation might also induce acetylcholine release.

Despite findings suggesting that nicotinic AChR activation directly affects neuroblast development, the mechanisms by which this occurs are not understood. Recent reports suggest that nicotine can regulate the synthesis and/or degradation of trophic factors, including platelet derived growth factor, tumor necrosis factor-α, and transforming growth factor-β , which can enhance or impede cell growth in transformed cell lines.[198,199] Alternatively, nicotinic AChRs may directly couple to mitogenic signaling events as shown in cell lines, as well as in primary retinal and hippocampal neurons.[200-202] Irrespective of the mechanisms involved, it appears that nicotine *per se* can directly modulate cerebellar development by affecting granule cell maturation. For this reason, recent suggestions that nicotine replacement therapy be used during pregnancy as a substitute for cigarette smoking, should be judiciously approached.[203] While this seems a prudent measure because the myriad products in cigarette smoke besides nicotine are likely to be far more adverse than nicotine alone, it might only a partial solution assuming nicotine itself effects neural maturation.

Conclusions

Despite findings that opiate drugs and/or nicotine can intrinsically affect the maturation and survival of isolated EGL cells in culture, caution should be used before generalizing these results to effects in the whole organism. In the absence of the complex cues normally present within the microenvironment of the developing brain, it is premature to speculate whether granule cells might respond similarly *in vivo*, or whether the untimely exposure to opiate drugs or nicotine during maturation might have similar influences on human cerebellar development. Moreover, pharmacodynamic differences in drug exposure make it challenging to generalize experimental findings from *in vivo* or *in vitro* animal models to human development.

An underlying assumption is that drug abuse impacts cerebellar maturation by modulating the degree and timing of ongoing developmental events. In addition to the potential for additive and synergistic interactions, pharmacodynamic differences in drug exposure (versus the neurochemical systems they mimic) potentially activate novel signaling events and genes. The response of a cell to a single drug such as heroin likely reflects the synergistic effect of heroin's actions through multiple signaling cascades and downstream effectors. Future studies are beginning to tackle this complexity using gene microarrays, proteomics, and new means of combinatorial analysis of complex data sets. The cerebellum, in general, and granule cells, in particular, which display highly delineated spatial and temporal patterns of development, will continue to provide an excellent model system to elucidate how drug abuse disrupts the CNS maturation.

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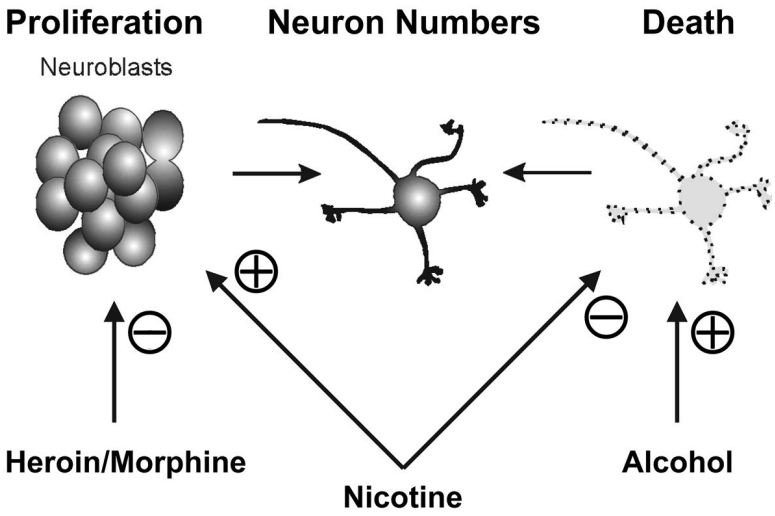

Figure 1. Summary of the principal effects of various substances on the production of granule neurons.

The number of granule neurons in the cerebellum is determined by two key developmental events— cell proliferation and programmed cell death. Drug abuse can independently affect each event.[101] Evidence suggests that alcohol, opiate drugs (heroin and morphine), and nicotine disrupt granule neuron numbers through differing mechanisms that modulate cell proliferation and/or death.

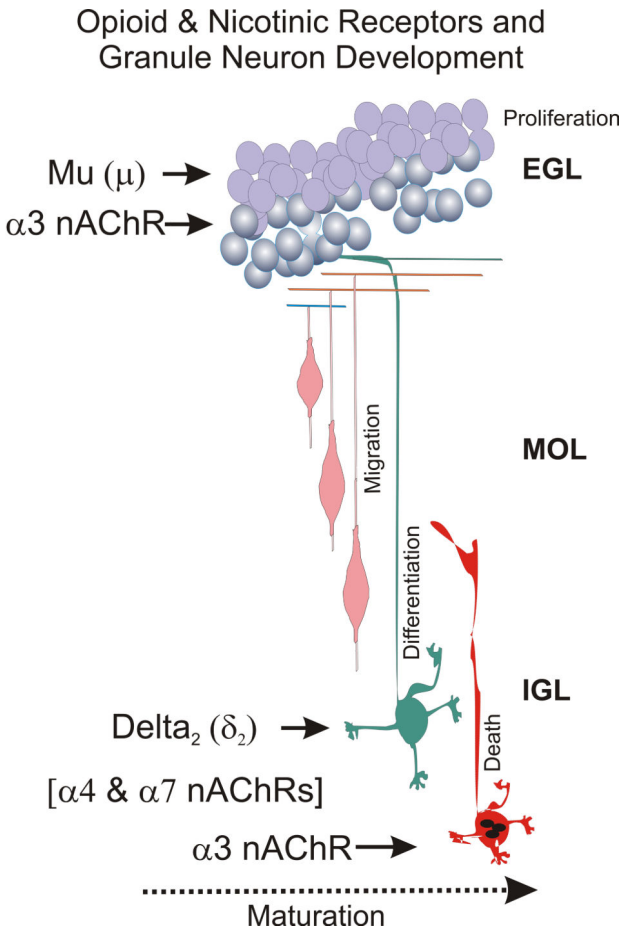

Figure 2. Summary: Opioid and nicotine actions during granule cell development.

Cerebellar granule neuron proliferation occurs in the external granular layer (EGL), a transient layer of proliferating cells that disappears in the adult. After cell division is completed, the neurons migrate past the molecular (MOL) and Purkinje cell (PC) layers to their adult positions in the internal granule layer (IGL) where they differentiate forming dendrites and synapses. Programmed cell death (apoptosis) can occur (perhaps by independent processes) in both the EGL and the IGL. Opiate drugs of abuse, such as heroin, inhibit cell replication through direct actions on μ opioid receptors, while δ2 opioid receptor agonists inhibit differentiation. Nicotine directly increases granule neuron numbers by independently increasing granule cell proliferation, while attenuating cell death. Both the mitogenic and antiapoptotic effects are likely mediated by α3 nicotinic AChR subunits. In contrast, α7 and α4 nAChR subunits (α4 & α7 nAChRs) are expressed later and potentially affect the maturation of more mature granule neurons, although there is no direct evidence for this at present.

Table 1

Effects of alcohol, opiates, and nicotine on granule and Purkinje cell development in the cerebellum

Substance	Target Cell Population	Effect (References)
Alcohol	Granule Neurons	Proliferation (↓ or no effect)[35,204]
		Differentiation* (Δ)[40] / Migration (Δ)[40,41,44]
		Death (↑)[38,45-50,54,66-69,73,204-206]
	Purkinje Cells	Proliferation (?)
		Differentiation (↓)[207]
		Death (↑) (or reduced cell numbers)[74,208,209]
Opioids	Granule Neurons	Proliferation (↓)[90,92,155]
		Differentiation (Δ)[34,88,92]
		Death (no effect)[92,155]
	Purkinje Cells	Proliferation (?)
		Differentiation (↓)[33,88,106]
		Death (↑)[33,210]
Nicotine	Granule Cells	Proliferation (↑)[180]
		Differentiation (?) (Δ synaptic function)[168,182,184,211,212]
		Death (↓)[180]
	Purkinje Cells	Proliferation (?)
		Differentiation (?) (Δ synaptic function)[185,213-217]
		Death (?)

Alcohol and drug effects on Purkinje cell maturation are noted because the profound interdependence of granule and Purkinje neurons during development.

*Differentiation is defined specifically as an alteration in the growth or complexity of axons and/or dendrites. *Key:* ↑ = *increased;* ↓ = *decreased;* A = *changed or disrupted; ? = uncertain*