Cerebellar Nucleus Development

16

Hong-Ting Kwok and Richard J. T. Wingate

Abstract

The cerebellar nuclei play a role in integrating cerebellar cortical output with inputs from other brain regions. Made up of a complex collection of both excitatory and inhibitory projection neurons as well as subtypes of interneurons, the cerebellar nuclei are developed from two germinal regions, the rhombic lip and the ventricular zone. In this chapter, we describe the developmental timeline of the cell types in relation to the development of the rest of the cerebellum, and how although the gross nuclei structures vary along the evolutionary tree, the cell types within are mostly conserved across evolution.

Keywords

Cerebellar nuclei · Development · Rhombic lip
Ventricular zone · Rhombomere 1

The cerebellar nuclei (CN) are the final output structures of the cerebellum, integrating inputs from the forebrain, brainstem, and spinal cord with the cerebellar cortical output from Purkinje cells. In contrast to the remarkably evolutionarily conserved connectivity between granule cells and Purkinje cells in the cerebellar cortex, the size, foliation, and number of CN vary between animals (1 in amphibians, 2 in reptiles and birds, and 3–5 in mammals) (Nieuwenhuys et al. 1998). In humans, there are four nuclei: the medial (fastigial), anterior and posterior interposed are classed as separate nuclei.

Historically, much of what is known concerning CN cell morphologies and neuronal circuitry comes from Golgi and Nissl preparations of the mammalian lateral nucleus observed by light and electron microscopy (Chan-Palay 1977). Neurotransmitter content and neuronal connectivity identified glutamatergic cells and three inhibitory cell classes. Recent single-cell RNA analysis combined with anatomical tracing identified that glutamatergic output cells are of two different types but confirmed that there are two glycinergic populations and a GABAergic inhibitory output neuron projecting to the inferior olive (Kebschull et al. 2020; Batini et al. 1992; Chen and Hillman 1993; Fredette et al. 1992) (Fig.16.1).

CN receive inhibitory projections from the Purkinje cells from the overlying cerebellar cortex in a broadly topographic manner. The lateral nuclei are innervated by the lateral cerebellum and medial nuclei by the medial vermis (Voogd and Glickstein 1998). CN also integrate collateral inputs from axons projecting from the pontine nucleus and inferior olive to the cerebellar cortex (Fig.16.1). The output of each CN is then directed to different central neural systems as determined by the pattern of their efferent connections (Larsell and Jansen 1972). Of these, the connection from the lateral nucleus to the ventrolateral thalamus is a uniquely mammalian adaptation and completes a closed-loop cortico-ponine-cerebellar relay circuit (Kelly and Strick 2003) that is heavily implicated in modulating higher cognitive function in humans (Schmahmann 2010).

H.-T. Kwok · R. J. T. Wingate (✉)
MRC Centre for Neurodevelopmental Disorders, King's College London, London, UK
e-mail: hongting_prekop@gis.a-star.edu.sg; richard.wingate@kcl.ac.uk

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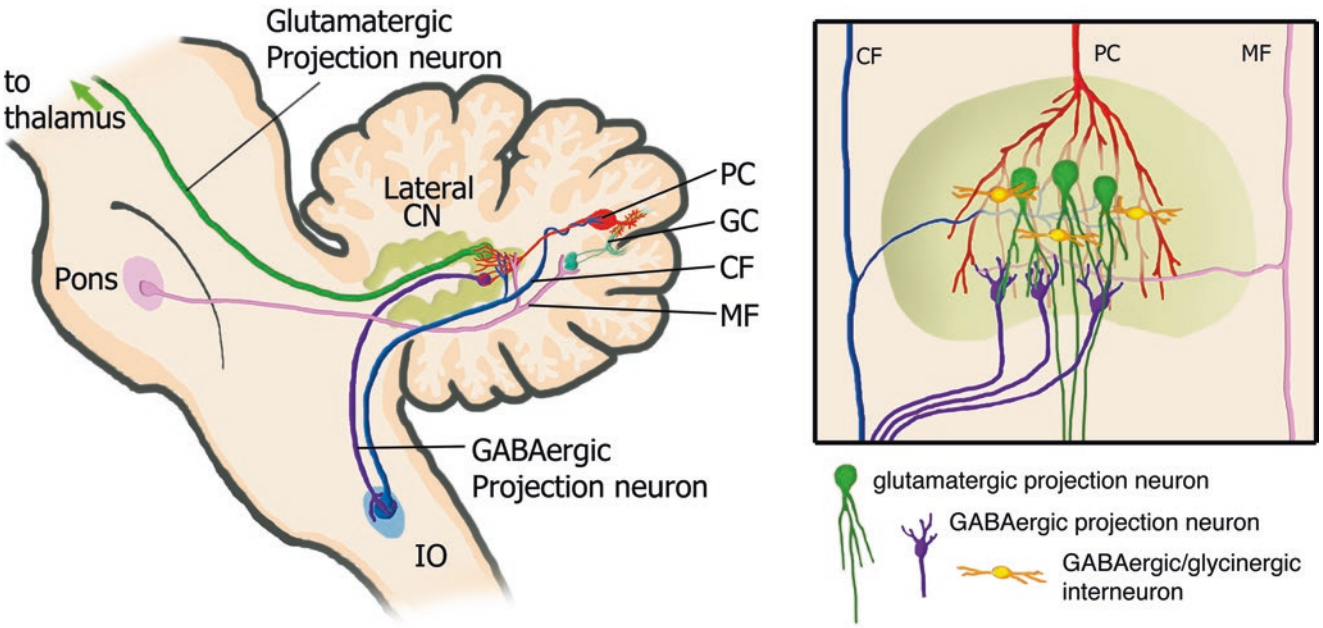

Fig. 16.1 Connections and circuitry of the human lateral nucleus. Left: The lateral CN receives input from Purkinje cells (PC) and innervates the thalamus, which in turn modulates cortical activity. The nucleus also receives climbing fibers (CF) collateral input from the inferior olive (IO), and mossy fibers (MF) collaterals, originating principally from the pons. Climbing and mossy fibers terminate on PC and granule cells (GC) in the cerebellar cortex, respectively. The CN also contains GABAergic projection neurons that send inhibitory signals to the IO. Right: Within the nucleus, each PC axon fans out into a cone that innervates a number of excitatory and GABAergic projection neurons. Small GABAergic and glycinergic interneurons provide local inhibition. Inputs from MF and CF collaterals run perpendicular to the afferent Purkinje cell axons [adapted from (Chan-Palay 1977)]

16.1 Concepts of CN Development Have Changed Markedly in Recent Years

Cell types of the cerebellum arise from two germinal regions within the most anterior neuromere of embryonic hindbrain, rhombomere 1: the ventricular zone (VZ) and the rhombic lip (RL). The VZ is a neuroepithelial zone that lines the dorsolateral part of the fourth ventricle, while the RL comprises the interface of this neuroepithelium with the roof plate of the fourth ventricle (Wingate 2001). Until the last decade, it was thought that all CN neurons originate from the VZ then migrate radially into the white matter (Altman and Bayer 1985a, b; Goldowitz and Hamre 1998). It is now known that CN neurons of different neurotransmitter types are born from both the RL and VZ.

Research by classical birth dating and genetic fate mapping has shown that all cerebellar excitatory neurons are derived from RL progenitors, specified by the expression of *Atoh1*, while inhibitory neurons arise from the VZ, where progenitors are specified by the early expression of *Ptf1a* (Hoshino et al. 2005). The bHLH proteins, *Ptf1a* and *Atoh1*, have both been shown to be necessary (Machold and Fishell 2005; Wang et al. 2005) and sufficient (Yamada et al. 2014) for the production of all GABAergic and glutamatergic neurons in the cerebellum, respectively (Fig.16.2). A small number of large glycinergic projection neurons, which are only found in the medial nucleus (Bagnall et al. 2009), are the exception, being potentially derived from the RL despite being inhibitory (Kebschull et al. 2020). The following description exemplifies CN development using the embryonic mouse model over its 21 days of gestation.

16.1.1 Glutamatergic Neurons are Born at the Rhombic Lip

Atoh1-expressing progenitor cells from the RL produce glutamatergic CN projection neurons between embryonic day (e)10–e12.5 prior to making granule cell precursors that populate the external granule layer (EGL) (Machold and Fishell 2005; Wang et al. 2005). From e12.5 to e14.5, the CN cells migrate from the RL across the dorsal surface of rhombomere 1 via the subpial rhombic lip migratory stream (RLS), then congregate at the nuclear transitory zone (NTZ) at the boundary of the cerebellar anlage (Fig.16.2). The NTZ is thought to be a transient differentiation zone (Altman and Bayer 1985a), where nuclear neurons are defined by specific, temporally restricted, developmental transcription factor profiles (Fink et al. 2006).

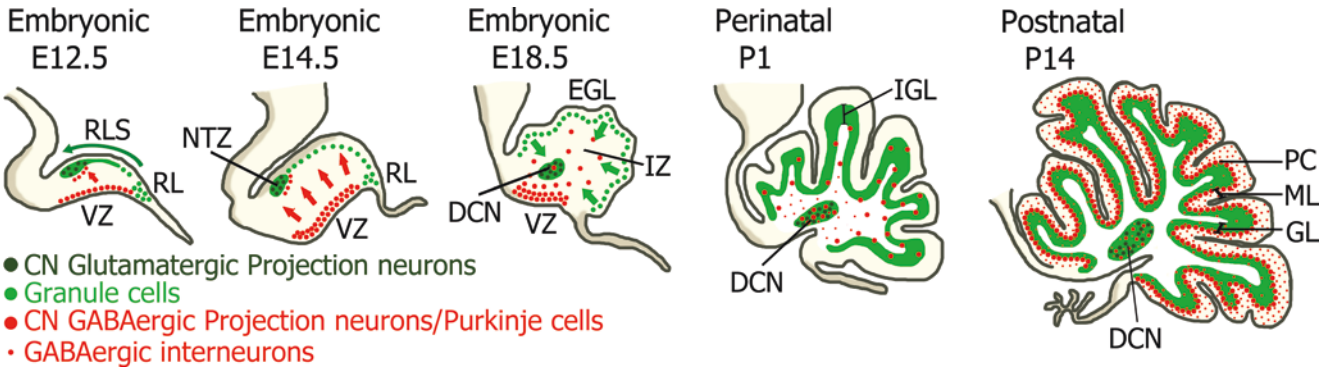

Fig. 16.2 Cerebellar nucleus neurons have a dual origin. Different neuronal subtypes in the cerebellum develop from separate germinal regions. Precursors continue to migrate, proliferate, differentiate, and mature postnatally. The CN glutamatergic cells form first at the RL and migrate via the nuclear transitory zone (NTZ). CN GABAergic projection neurons are also born early and migrate to the ventral part of the NTZ. The RL then gives rise to granule cell precursors, which form the external granule layer (EGL) that later migrates in to form the internal granule layer (IGL). All cortical GABAergic cells originate from the VZ, migrate into the intermediate zone (IZ), and finally to the molecular (ML) and granule (GL) cell layers

The CN are born in a lateral to medial sequence subsequent to the first-born RL derivatives, which become extracerebellar neurons (Machold and Fishell 2005). Like these extra-cerebellar neurons, nuclear cells in the lateral nucleus of mammals express the LIM-homeodomain gene *Lhx9* (Wang et al. 2005; Green and Wingate 2014). Both these early populations project to the thalamus suggesting a role for Lhx9 in specifying axonal projection (Green and Wingate 2014). Subsequently, RL-derived projection neurons of the interposed and medial nuclei are defined by their expression of *Tbr2* and *Tbr1*, respectively (Fink et al. 2006; Engelkamp et al. 1999; Landsberg et al. 2005) and extend axons to various hindbrain, midbrain, and ventral diencephalic targets.

From e14.5 to e16.5, CN cells in the NTZ descend into the white matter. It is unclear whether this is due to active migration toward the VZ (Altman and Bayer 1985a) or displacement by gross morphogenic changes to cerebellar shape as granule cell precursors in the EGL proliferate to produce the most abundant neuronal population in the brain.

16.1.2 GABAergic Neurons are Derived from the Ventricular Zone

Fate-mapping studies indicate that GABAergic CN cells are derived from *Ptf1a*-positive precursors in the VZ in two phases (Hoshino et al. 2005). First, GABAergic neurons that project long axons from the CN to the inferior olive (Mugnaini and Oertel 1985; Ruigrok 1997) are born within a distinct temporal window alongside Purkinje cells (e10.5–e12.5), both characterized by the expression of *Olig2*. In synchrony with the glutamatergic projection neurons being derived from the RL, these nucleo-olivary neurons accumulate adjacent and inferior to the NTZ before descending to their destination (Prekop et al. 2018).

From e13.5, *Olig2* is downregulated and subsequent populations of GABAergic neurons express *Gsx1* (Seto et al. 2014) and Pax2 (Maricich and Herrup 1999; Weisheit et al. 2006). *Pax2*-positive precursors proliferate within the white matter through to P15 and migrate radially to sequentially form various GABAergic interneuron populations: first the CN interneurons, then Golgi cells of the granule cell layer, and finally basket and stellate cells of the molecular layer (Leto et al. 2006). A growing body of evidence shows that specification is controlled post-mitotically by factors in the local microenvironment (Leto et al. 2009; Grimaldi et al. 2009; Zordan et al. 2008), although their identity, and the contribution, if any, of intrinsic cues are still largely undefined.

16.2 Future Studies will Need to Address Fine-Grain Patterning of Different Nuclei

Our current understanding outlines basic principles of CN development in terms of progenitor zones, temporal patterning, and the function of a few key transcription factors. Recent studies have illustrated how the discovery of new molecular and genetic markers has allowed fate mapping of distinct cell types. Detailed studies of cell organization within the lateral nucleus have revealed intricate cell arrangements and alignment of projections along a polarized axis within the nucleus (Chan-Palay 1977). In addition, single cell and spatial omics analysis have made possible deeper interrogation of developing cell types, as well as postulate how conserved cell types may form an archetypal nucleus

that has duplicated with variation over evolution. This has shown that while the ventricular zone-derived inhibitory cells are genetically uniform across nuclei, it is the rhombic lip derived glutamatergic populations that confer genetic diversity on the cerebellar nuclei (Kebschull et al. 2020).

How neuroblasts migrate, differentiate, and successfully form functional circuits are important open questions. The identity of cues that shape CN circuits will be important targets for future research. This will also help in assessing the impact of CN dysgenesis on a broad spectrum of cerebellar disorders that can produce both classical motor symptoms and an emerging range of cognitive effects in syndromes such as Autistic Spectrum Disorder and Joubert Syndrome (Schmahmann 2010; Wang et al. 2014; Holroyd et al. 1991).

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