The Olivocerebellar Tract

6

Yuanjun Luo and Izumi Sugihara

Abstract

Neurons in the inferior olive nucleus, the sole origin of cerebellar climbing fibers, project their axons to the cerebellum through the olivocerebellar tract. A single olivocerebellar axon gives rise to multiple climbing fibers (about seven in rats), which often terminate into a single longitudinal compartment defined by the cerebellar cortex's longitudinal striped molecular expression pattern. According to an intriguing topographic relationship, axons originating from a subarea of the inferior olive project to a particular compartment. As a result of this topographic arrangement, the olivocerebellar projection relays synchronous activity of the electrically coupled adjacent inferior olive neurons to complex spike firing of Purkinje cells in a longitudinal compartment. Olivocerebellar axons show a dynamic morphogenetic process. An immature axon has abundant terminal branches that innervate many Purkinje cells. Several terminal branches (climbing fibers) grow to eventually establish a powerful one-to-one synaptic connection between a single climbing fiber terminal and a single target Purkinje cell. Furthermore, these axons are capable of strong compensatory re-innervation after lesion, even in the adult.

6.1 Introduction

The olivocerebellar tract is the axonal path of inferior olive neurons. The olivocerebellar axons form the climbing fiber projection, one of the two major afferent systems in the cerebellar cortex. The olivocerebellar climbing fiber projection system is unique in morphological, physiological, and developmental aspects, making a sharp contrast with the mossy fiber system, the other of the two major afferent systems of the cerebellum. The olivocerebellar projection system contributes significantly to characterize the functional organization of the cerebellum. This short article summarizes the olivocerebellar projection's essential morphological, physiological, and developmental characteristics to show its uniqueness based on relevant studies, including ours.

6.2 Morphology of Single Olivocerebellar Axons

The inferior olive nucleus, located in the caudoventral medulla, is a complex multi-lamella structure packed with small neurons with round somata and curved dendrites. Except for the negligible number of scattered GABAergic neurons, all neurons in the inferior olive nucleus project to the cerebellum, terminating as climbing fibers. The inferior olive is the sole origin of climbing fibers. Therefore, the inferior olive is a critical node in the input circuitry of the cerebellum.

The olivocerebellar tract is the bundle of axons of the inferior olive neurons projecting to the cerebellar cortex. The axons run medially, crossing the midsagittal plane and continuing through or above the contralateral inferior olive, before entering the white matter under the lateral surface of the medulla that connects to the inferior cerebellar peduncle (Fig. 6.1a, Sugihara et al. 1999). Before entering the cerebellum through the inferior cerebellar peduncle, the axons that terminate in the vermis pass through the most rostral part of the cerebellar peduncle dorsal to the superior cerebellar peduncle intermingled with the uncinate fasciculus and the ventral spinocerebellar tract (arrowhead in Fig. 6.1). Other olivocerebellar axons pass through the conventional inferior cerebellar peduncle.

Y. Luo · I. Sugihara (✉)
Department of Systems Neurophysiology and Center for Brain Integration Research, Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, Tokyo, Japan
e-mail: Isugihara.phy1@tmd.ac.jp

D. L. Gruol et al. (eds.), *Essentials of Cerebellum and Cerebellar Disorders*, https://doi.org/10.1007/978-3-031-15070-8_6

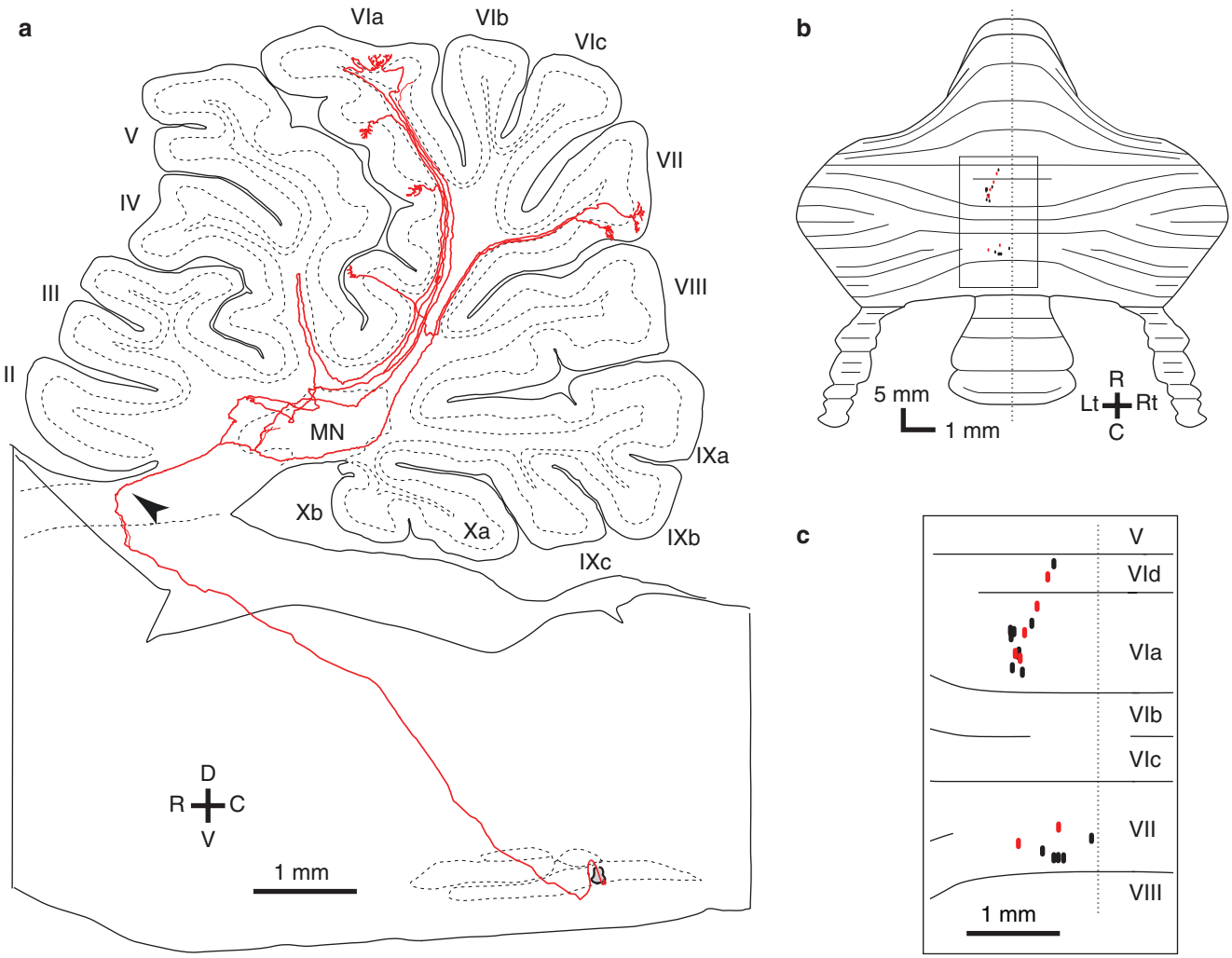

Fig. 6.1 Axonal path of a single olivocerebellar axon. (**a**) Lateral view of a trajectory of a reconstructed single olivocerebellar axon labeled by localized injection of biotinylated dextran amine in the rat. This axon originated from the medial and caudal part of the medial accessory olive, a subnucleus of the inferior olive, and terminated in lobules VI and VII in the vermis. Arrowhead indicates the most rostral part of the inferior cerebellar peduncle. (**b**) Distribution of climbing fibers originating of this axon (red) and other axons labeled (black) mapped in the unfolded scheme of the rat cerebellar cortex. (**c**) Magnified drawing of the area surrounded by the square in (**b**). *II–Xb* lobule II—lobule Xb, *C* caudal, *IO* inferior olive, *Lt* left, *MN* medial nucleus, *R* rostral, *Rt* right

Upon entering the cerebellum, each axon gives rise to collaterals to the deep cerebellar nuclei and branches into multiple (seven on average in rat) axons, each of which terminates on a single adult Purkinje cell as a climbing fiber. Thus, a "climbing fiber" discovered by Ramón y Cajal (1911) is the terminal arbor of one of a number of branches of the olivocerebellar axon. Besides giving rise to nuclear collaterals and cortical branches that terminate as climbing fibers, olivocerebellar axons also give rise to several thin collaterals, mainly terminating in the granular layer with a small number of swellings. Synaptic contact and functional significance of these collaterals are not well clarified (Sugihara et al. 1999). The multiple climbing fibers originating from a single axon are usually, but not always, distributed in a narrow longitudinal band-shaped area (Fig. 6.1b, c, Sugihara et al. 2001) inside one zebrin stripe (later section). The olivocerebellar axon's longitudinal projection pattern contrasts with the transversely wide projection pattern of mossy fiber axons (Biswas et al. 2019).

6.3 Topography in the Olivocerebellar Tract

The olivocerebellar projection has a topographic arrangement. Thus, the inferior olive subdivides into many subareas, usually a portion of a single lamella (Fig. 6.2a). Neurons in each subarea of the inferior olive project topographically to a par-

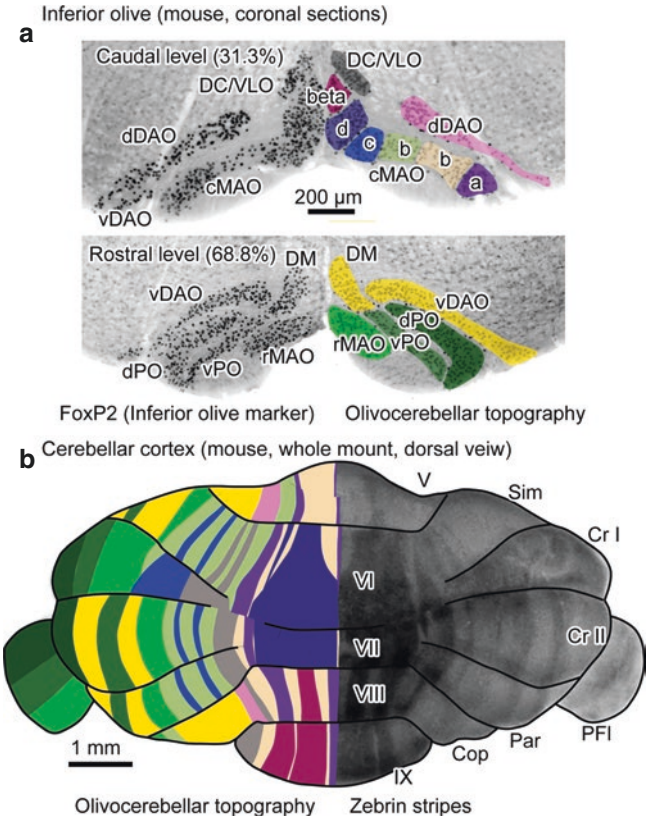

Fig. 6.2 Aldolase C stripe-linked topography of the olivocerebellar projection. (**a**) Subareas of the inferior olive. Two coronal sections of the inferior olive of the mouse, labeled with immunostaining of FoxP2, a marker molecule of the nucleus of inferior olive neurons (Fujita and Sugihara 2012) at the caudal and rostral levels (reversed epifluorescence image). The percentile indicates the relative caudorostral levels within the whole inferior olive. The superimposition of colors on the right side shows the subareas of the inferior olive. (**b**) Dorsal view of the whole-mount preparation of the Aldoc-Venus mouse (Fujita et al. 2014), in which aldolase C (zebrin) stripes are visible with fluorescence (reversed epifluorescence image). On the left side, the cerebellar cortex is divided into multiple zonal areas (colored) that receive the topographic olivocerebellar projection from subareas of the inferior olive. The boundaries of colored stripes mostly match with the boundaries of aldolase C stripes. The color-coding indicates the topographic relationship between A and B. The dark gray area in A and the gray area in B do not have corresponding parts appearing in the other panel. Based on Sugihara and Shinoda (2004), Sugihara and Quy (2007) and Fujita et al. (2020). *V–IX* lobule V–lobule IX, *a, b, c, d* subnucleus a, b, c, d of the caudal part of the medial accessory olive, *beta* subnucleus beta, *DM* dorsomedial subnucleus, *DC/VLO* dorsal cap, and ventrolateral outgrowth subnucleus, *dDAO* dorsal fold of the dorsal accessory olive, *dPO* dorsal lamella of the principal olive, *rMAO* rostral part of the medial accessory olive, *vPO* ventral lamella of the principal olive, *Sim* simple lobule, *Cr I* crus I, *Cr II* crus II, *Par* paramedian lobule, *Cop* copula pyramidis, *PFl* paraflocculus

ticular subarea in the cerebellar nuclei and a specific striped subarea in the cerebellar cortex (Sugihara and Shinoda 2004, 2007). These subareas in the cortex and nuclei are topographically connected to each other by the corticonuclear Purkinje cell projection (Sugihara et al. 2009). The specific subarea in the cerebellar nuclei also topographically projects to the particular subarea in the inferior olive (Ruigrok and Voogd 1990). As a whole, a triangular topographic loop of neuronal connections forms among subareas in the inferior olive, cerebellar cortex, and cerebellar nuclei. Each set of topographically connected subareas in the cerebellar cortex, cerebellar nuclei, and inferior olive is designated as a cerebellar module (Ruigrok 2011). A standing question of how many modules constitute the entire cerebellum remains. Conventionally, the basic six modules (A, B, C1, C2, C3, and D) have been recognized (Voogd and Bigaré 1980). However, most of these modules have been further subdivided into smaller modules (Ruigrok 2011; Sugihara et al. 2009). In addition, the flocculus and nodulus have distinct modules (Sugihara et al. 2004). Most cerebellar modules are consistent with the cortical compartments defined by the molecular expression profile in Purkinje cells (Sugihara and Shinoda 2004, Sugihara et al. 2009). Aldolase C (zebrin II, or just "zebrin," Brochu et al. 1990), which is the representative of such molecules, is highly expressed in Purkinje cells arranged in tens of alternate longitudinal stripes in the cerebellar cortex (Fig. 6.2b, right). The topographic relationship between the subarea of the inferior olive and aldolase C stripes has been well identified (color-coded in Fig. 6.2), although details may still be revised in the future. In the cerebellar nuclei, to which the collaterals of olivocerebellar axons project topographically, the arrangement of subareas (or modules) is different from that in the cerebellar cortex. Subareas linked with aldolase C-positive and -negative compartments are all located in the caudoventral and rostrodorsal parts of the cerebellar nuclei, respectively (Sugihara and Shinoda 2007). Output neurons in different subareas of the cerebellar nuclei linked with aldolase C stripes generally project to distinct targets and are involved in different cerebellar functions (Fujita et al. 2020).

6.4 Physiological Properties

The inferior olive neurons show the oscillatory fluctuation of membrane potential at about 10 Hz (Llinás and Yarom 1986). This activity synchronizes among nearby neurons through dendro-dendritic gap junctions (Llinás and Yarom 1986; Long et al. 2002). Excitatory input to the inferior olive, which mainly originates from the somatosensory and vestibular systems in the medulla and spinal cord and visual, corticofugal, and other systems in the midbrain and mesodiencephalic junction (see Sugihara and Shinoda 2004), may reset the oscillatory rhythm to evoke firing (Leznik and Llinás 2005). Olivary cells may fire at the peak of the oscillation of one action potential or a few action potentials in a burst. The firing of an action potential (or a brief burst of action potentials) occurs as a solitary event or in sequence with about 100-ms intervals (Marthy et al. 2009). On average, the firing frequency of the olivary neuron is about 1 Hz (Eccles et al. 1966).

The firing of olivary neurons is conveyed to the axon terminals, i.e., climbing fiber terminals, with a conduction time of approximately 4 ms (in rat, Sugihara et al. 1993). An action potential (or a brief burst of action potentials) in the climbing fiber produces a complex spike response in target Purkinje cells (Eccles et al. 1966). In addition, olivocerebellar axon collaterals elicit an excitatory effect in the cerebellar nuclei (Blenkinsop and Lang 2011). However, its effect on the granular layer is yet unclear.

Since adjacent inferior olive neurons generally project to a narrow longitudinal striped area in the cerebellar cortex, it often matches with a single aldolase C stripe (Sugihara et al. 2007). Because of this property in the olivocerebellar projection, Purkinje cells arranged in the longitudinal band (width = ~0.25 mm) tend to fire complex spikes synchronously in awake and anesthetized states (Sasaki et al. 1989; Lang et al. 1999). The band of complex spike synchrony generally matches with a single aldolase C stripe (Sugihara et al. 2007). Functionally, climbing fiber inputs in each aldolase C stripe are activated in particular timing/aspects during the sensorimotor behavior of animals (Horn et al. 2010; Tsutsumi et al. 2019). This synchronous complex spike firing of Purkinje cells may be functionally important to form cerebellar output in the cerebellar nuclei (Blenkinsop and Lang 2011).

6.5 Development of the Olivocerebellar Projection

The immature olivocerebellar axonal projection is formed in the late embryonic stage when Purkinje cells form clusters before settling into striped compartments in rats and mice (Fujita et al. 2012). A basic topographic projection pattern appears in the olivocerebellar bundle at this stage (Chédotal and Sotelo 1992). Axonal terminals form a delicate plexus with abundant branching known as the creeper terminal (Sugihara 2005). In the second postnatal week, many axonal branches disappear, leaving only those that begin to form a dense arbor around a single Purkinje cell soma (nest terminal). The nest terminals grow to an entire climbing fiber terminal in the following few weeks.

The above process of climbing fiber development leads to the establishment of a one-to-one synaptic connection between a single climbing fiber terminal and a single target Purkinje cell. A loss of granule cells caused by X-ray irradiation or other procedures and some genetic mutations prevent the normal development of climbing fiber morphology (Sugihara et al. 2000). In these situations, multiple climbing fibers originating from one olivocerebellar axon (pseudo-multiple innervation) of different olivocerebellar axons (true multiple innervations) may remain to innervate a single Purkinje cell (impairment of one-to-one innervation, Sugihara et al. 2000).

6.6 Plasticity of Olivocerebellar Axons

Since the average olivocerebellar projection is nearly exclusively contralateral, an increase of ipsilateral projection indicates a plastic change in the projection. Such plastic change is seen after a unilateral cut of the cerebellar peduncle in the neonatal stage in rats (Sugihara et al. 2003). A similar transcommissural olivocerebellar projection to the ipsilateral cerebellum is seen even in adults after administering substances that can facilitate axonal plasticity (Dixon and Sherrard 2006).

Semitotal lesion of the inferior olive by neurotoxin 3-aminopyridine (3-AP) induces axonal sprouting of remaining olivary neurons to compensate for the loss of many olivocerebellar axonal terminals (Rossi et al. 1991). Axonal sprouting occurs only in their terminal portions, mainly in the terminal arbor of climbing fibers and possibly also at the terminal of thin collaterals in the granular layer and cerebellar nuclei. However, no axonal sprouting from the stem axon in the cerebellar white matter was evident, at least in adults (Aoki and Sugihara 2012).

6.7 Conclusion

Our current knowledge of the olivocerebellar projection's morphological, physiological, and developmental aspects are summarized above. The olivocerebellar projection is an essential component of the cerebellar system. The projection is well organized at the level of single axons and at the level of topographic compartmentalization of the whole cerebellar cortex. The input from the olivocerebellar system produces significant effects on the activity of Purkinje cells and the output of neurons of the cerebellar nucleus. To consider the significance of such effect in various cerebellar functions, the general morphological property of the olivocerebellar system as summarized here would be necessary.

References

Aoki H, Sugihara I (2012) Morphology of single olivocerebellar axons in the denervation–reinnervation model produced by subtotal lesion of the rat inferior olive. Brain Res 1449:24–37

Biswas MS, Luo Y, Sarpong GA, Sugihara I (2019) Divergent projections of single pontocerebellar axons to multiple cerebellar lobules in the mouse. J Comp Neurol 527:1966–1985

Blenkinsop TA, Lang EJ (2011) Synaptic action of the olivocerebellar system on cerebellar nuclear spike activity. J Neurosci 31:14708–14720

Brochu G, Maler L, Hawkes R (1990) Zebrin II: a polypeptide antigen expressed selectively by Purkinje cells reveals compartments in rat and fish cerebellum. J Comp Neurol 291:538–552

Chédotal A, Sotelo C (1992) Early development of olivocerebellar projections in the fetal rat using CGRP immunocytochemistry. Eur J Neurosci 4:1159–1179

Dixon KJ, Sherrard RM (2006) Brain-derived neurotrophic factor induces post-lesion transcommissural growth of olivary axons

that develop normal climbing fibers on mature Purkinje cells. Exp Neurol 202:44–56
Eccles JC, Llinás R, Sasaki K (1966) The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. J Physiol 182:268–296
Fujita H, Sugihara I (2012) FoxP2 expression in the cerebellum and inferior olive: development of the transverse stripe-shaped expression pattern in the mouse cerebellar cortex. J Comp Neurol 520:656–677
Fujita H, Morita N, Furuichi T, Sugihara I (2012) Clustered fine compartmentalization of the mouse embryonic cerebellar cortex and its rearrangement into the postnatal striped configuration. J Neurosci 32:15688–15703
Fujita H, Aoki H, Ajioka I, Yamazaki M, Abe M, Oh-Nishi A, Sakimura K, Sugihara I (2014) Detailed expression pattern of aldolase C (Aldoc) in the cerebellum, retina and other areas of the CNS studied in Aldoc-Venus knock-in mice. PLoS One 9:e86679
Fujita H, Kodama T, du Lac S (2020) Modular output circuits of the fastigial nucleus for diverse motor and nonmotor functions of the cerebellar vermis. Elife 9:e58613
Horn KM, Pong M, Gibson AR (2010) Functional relations of cerebellar modules of the cat. J Neurosci 30:9411–9423
Lang EJ, Sugihara I, Welsh JP, Llinás R (1999) Patterns of spontaneous complex spike activity in the awake rat. J Neurosci 19:2728–2739
Leznik E, Llinás R (2005) Role of gap junctions in synchronized neuronal oscillations in the inferior olive. J Neurophys 94:2447–2456
Llinás R, Yarom Y (1986) Oscillatory properties of guinea-pig inferior olivary neurones and their pharmacological modulation: an in vitro study. J Physiol 376:163–182
Long MA, Deans MR, Paul DL, Connors BW (2002) Rhythmicity without synchrony in the electrically uncoupled inferior olive. J Neurosci 22:10898–10905
Marthy A, Ho SSN, Davie JT, Duguid IC, Clark BA, Häusser M (2009) Encoding of oscillations by axonal bursts in inferior olive neurons. Neuron 62:388–399
Ramón y Cajal S (1911) Histologie du système nerveux de l'homme et des verébrés, vol 2. Maloine, Paris
Rossi F, Wiklund L, Van der Want JJL, Strata P (1991) Reinnervation of cerebellar Purkinje cells by climbing fibers surviving a subtotal lesion of the inferior olive in the adult rat. I. Development of new collateral branches and terminal plexuses. J Comp Neurol 308:513–535
Ruigrok TJ (2011) Ins and outs of cerebellar modules. Cerebellum 10:464–474
Ruigrok TJ, Voogd J (1990) Cerebellar nucleo-olivary projections in the rat: an anterograde tracing study with *Phaseolus vulgaris* leucoagglutinin (PHA-L). J Comp Neurol 298:315–333
Sasaki K, Bower JM, Llinás R (1989) Multiple Purkinje cell recording in rodent cerebellar cortex. Eur J Neurosci 1:572–586
Sugihara I (2005) Microzonal projection and climbing fiber remodeling in single olivocerebellar axons of new born rats at postnatal days 4–7. J Comp Neurol 487:93–106
Sugihara I, Quy PN (2007) Identification of aldolase C compartments in the mouse cerebellar cortex by olivocerebellar labeling. J Comp Neurol 500:1076–1092
Sugihara I, Shinoda Y (2004) Molecular, topographic and functional organization of the cerebellar cortex: a study with combined aldolase C and olivocerebellar labelling. J Neurosci 24:8771–8785
Sugihara I, Shinoda Y (2007) Molecular, topographic and functional organization of the cerebellar nuclei: analysis by three-dimensional mapping of the olivonuclear projection and aldolase C labeling. J Neurosci 27:9696–9710
Sugihara I, Lang EJ, Llinás R (1993) Uniform olivocerebellar conduction time underlies Purkinje cell complex spike synchronicity in the rat cerebellum. J Physiol Lond 470:243–271
Sugihara I, Wu H-S, Shinoda Y (1999) Morphology of single olivocerebellar axons labeled with biotinylated dextran amine in the rat. J Comp Neurol 414:131–148
Sugihara I, Bailly Y, Mariani J (2000) Olivocerebellar climbing fibers in the granuloprival cerebellum: morphological study of individual axonal projections in the X-irradiated rat. J Neurosci 20:3745–3760
Sugihara I, Wu H-S, Shinoda Y (2001) The entire trajectories of single olivocerebellar axons in the cerebellar cortex and their contribution to cerebellar compartmentalization. J Neurosci 21:7715–7723
Sugihara I, Lohof AM, Letellier M, Mariani J, Sherrard RM (2003) Post-lesion transcommissural growth of olivary climbing fibres creates functional synaptic microzones. Eur J Neurosci 18:3027–3036
Sugihara I, Ebata S, Shinoda Y (2004) Functional compartmentalization in the flocculus and the ventral dentate and dorsal group y nuclei: an analysis of single olivocerebellar axonal morphology. J Comp Neurol 470:113–133
Sugihara I, Marshall SP, Lang EJ (2007) Relationship of complex spike synchrony bands and climbing fiber projection determined by reference to aldolase C compartments in crus IIa of the rat cerebellar cortex. J Comp Neurol 501:13–29
Sugihara I, Fujita H, Na J, Quy PN, Li BY, Ikeda D (2009) Projection of reconstructed single Purkinje cell axons in relation to the cortical and nuclear aldolase C compartments of the rat cerebellum. J Comp Neurol 512:282–304
Tsutsumi S, Hidaka N, Isomura Y, Matsuzaki M, Sakimura K, Kano M, Kitamura K (2019) Modular organization of cerebellar climbing fiber inputs during goal-directed behavior. eLife 8:e47021
Voogd J, Bigaré F (1980) Topographical distribution of olivary and corticonuclear fibers in the cerebellum: a review. In: Courville J, de Montigny C, Lamarre Y (eds) The inferior olivary nucleus anatomy and physiology. Raven Press, New York, pp 207–234