Biochemical and Biophysical Research Communications 449 (2014) 169–174

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Inhibitory effects of draxin on axonal outgrowth and migration of precerebellar neurons

M. Asrafuzzaman Riyadh [a,b], Yohei Shinmyo [a,*], Kunimasa Ohta [a], Hideaki Tanaka [a,†]

[a] Division of Developmental Neurobiology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan
[b] Stem Cell-Based Tissue Regeneration Research and Education Unit, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-0811, Japan

ARTICLE INFO

*Article history:*
Received 2 May 2014
Available online 14 May 2014

*Keywords:*
Draxin
Precerebellar neurons
Axon outgrowth
Migration

ABSTRACT

The rhombic lip, a dorsal stripe of the neuroepithelium lining the edge of the fourth ventricle, is the site of origin of precerebellar neurons (PCN), which migrate tangentially towards the floor plate. After reaching the floor plate, they project their axons to the cerebellum. Although previous studies have shown that the guidance molecules Netrin/DCC and Slit/Robo have critical roles in PCN migration, the molecular mechanisms underlying this process remain poorly understood. Here, we report that draxin, a repulsive axon guidance protein, is involved in PCN development. We found that *draxin* is expressed in the rhombic lip and migratory stream of some PCN in the developing hindbrain of mice. In addition, draxin inhibited neurite outgrowth and nuclei migration from rhombic lip explants. These results suggest that draxin functions as a repulsive guidance cue for PCN migration. However, we observed no significant differences in PCN distribution between *draxin*$^{-/-}$ and wild type embryos. Thus, draxin and other axon guidance cues may have redundant roles in PCN migration.

© 2014 Elsevier Inc. All rights reserved.

## 1. Introduction

In the development of the central nervous system, there are two main modes of neural migration; radial migration and tangential migration [1]. In vertebrates, hindbrain precerebellar neurons (PCN) represent an excellent model of tangential migration [2]. PCN consist of neurons that will form the external cuneate nucleus (ECN), the lateral reticular nucleus (LRN), the inferior olivary nucleus (ION), and the pontine nucleus (PN). All originate from the germinative neuroepithelium in the caudal hindbrain called the rhombic lip (RL) [3–5]. Although all PCN originate from the RL, they have a distinct temporal pattern of birthdates. They also differ according to migratory routes: PCN that will form the ION migrate through the submarginal stream, while those forming the LRN and ECN migrate through the marginal stream [3,4,6,7].

During their tangential migration, all PCN first extend a process that leads the way, directing the migratory route, until they reach and cross the floor plate [6]. The nuclei then translocate inside this leading process [8,9]. Upon reaching the floor plate, PCN exhibit distinct behaviors. ION neurons stop while their leading processes extend to the contralateral cerebellum. In contrast, LRN and ECN neurons keep moving across the floor plate until reaching their proper destination in the contralateral rhombencephalon. The floor plate exerts considerable influence on migrating PCN by expressing chemotropic factors. Several studies have revealed that *Netrin*-1, through the deleted in colorectal cancer (DCC) receptor, functions as an attractive cue in the migration of several types of PCN [9–16]. Slit-Robo signaling also plays an important role in directing PCN to cross the midline [17,18].

We previously reported that draxin, a chemorepulsive axon guidance molecule, has critical roles in formation of forebrain commissures and spinal cord commissure [19]. Indeed, draxin inhibits axonal outgrowth from several types of neurons, including spinal cord commissural [19], tectal [20], neocortical [19] and lateral olfactory bulb [21] neurons *in vitro*. In this study, we have focused on draxin functions in the migration of PCN. The expression of *draxin* in the rhombic lip at different developmental stages suggests that draxin might have a role in the migration of PCN. We investigated draxin activity for migratory potential and axon outgrowth of PCN *in vitro*, and analyzed different types of PCN migration in wild type and *draxin*$^{-/-}$ littermate.

* Corresponding author. Present address: Department of Biophysical Genetics, Graduate School of Medical Sciences, Kanazawa University, Ishikawa 920-8640, Japan. Fax: +81 76 234 4274.
*E-mail address:* shinmyo@med.kanazawa-u.ac.jp (Y. Shinmyo).
† Deceased.

http://dx.doi.org/10.1016/j.bbrc.2014.05.013
0006-291X/© 2014 Elsevier Inc. All rights reserved.

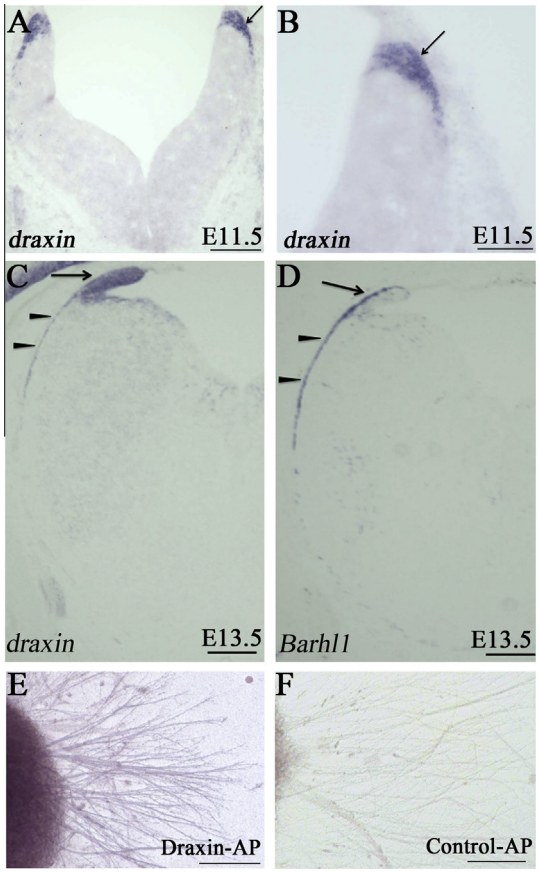


**Fig. 1.** Expression of *draxin* mRNA in the developing hindbrain. *Draxin* mRNA expression was revealed by *in situ* hybridization in coronal sections of wild type hindbrain at E11.5 (A and B) and E13.5 (C). *Draxin* was strongly expressed in the rhombic lip (RL, arrow) at E11.5 (A, higher magnification B). At E13.5, *draxin* expression was evident in the RL (arrow, C) and in the migratory stream of ECN/LRN neurons (arrowheads, C). The migratory stream (arrowheads) of LRN/ECN neurons is shown in the adjacent section using *Barhl1* expression (D). draxin–AP protein bound to the neurites originating from E11.5 RL explants (E), but control–AP protein did not (F), indicating the presence of draxin receptor/s in the neurites. Scale bars; A, C, D, 200 μm; B, E, F, 100 μm.

## 2. Materials and methods

### 2.1. Mice

All animals were treated according to protocols approved by the Committee on Animal Research at the University of Kumamoto. The procedure to generate *draxin* knockout (*draxin*$^{-/-}$) mice was described previously [19]. Mice heterozygous for *draxin* were crossed to generate *draxin* homozygous and wild type littermates, which were used for phenotype analysis. Appearance of the vaginal plug was designated as embryonic day 0.5 (E0.5).

### 2.2. Histological analyses and in situ hybridization

For histological analyses, timed pregnant mice, at the appropriate embryonic age, were used. Immunohistochemistry on brain sections was performed according to a previously described protocol [22], using a rabbit primary antibody against Forkhead box protein P2 (FoxP2, 1:1000; Abcam).

We used a digoxigenin-labeled (DIG) antisense RNA probe for *draxin* to assess *draxin* expression patterns in the hindbrain and a digoxigenin-labeled antisense RNA probe for *Barhl1* to identify LRN and ECN neurons. We used standard protocols for *in situ* hybridization, as described previously [19,23].

### 2.3. Preparation of control and draxin-alkaline phosphatase conditioned medium

To prepare control and draxin–alkaline phosphatase (draxin–AP) conditioned medium, cDNAs encoding chick draxin–AP conjugate and unconjugated AP (control–AP) tags were used, according to previously described protocols [19]. Synthesis of proteins was verified with western blot using an anti-chicken draxin monoclonal antibody.

### 2.4. Explant cultures and Draxin–AP binding

RL explants were collected from E11.5 and E12.5 wild type embryos as previously described [9] and cultured in Matrigel (BD Bioscience). Briefly, explants were embedded in Matrigel solution and allowed to set for 30 min. Explants were then cultured using neurobasal supplemented media containing either control–AP or draxin–AP, for 72 h at 37 °C in 5% $CO_2$. Neurobasal supplemented media composed of neurobasal medium, B27, glutamax-I, and penicillin/streptomycin, 1:1 ratio (Gibco® Life Technologies), For co-culture experiments, RL explants were co-cultured with HEK-293 cell aggregates transfected with either an empty vector or with cDNA encoding mouse draxin, as described previously [19,24]. Explants and cell aggregates were embedded in Matrigel 200–300 μm apart and cultured using neurobasal supplemented media for 60 h at 37 °C in 5% $CO_2$. For both bath and co-cultures, three independent experiments were performed, with at least five explants used in each condition.

To visualize neuronal processes, cultures were fixed for 1 h in 4% paraformaldehyde, rinsed several times, and incubated with a neuronal specific anti-β-III tubulin primary antibody (R&D systems, 1:1000). 4′,6-diamidino-2-phenylindole (DAPI) staining was used to visualize cell nuclei. Photographs were taken using an inverted fluorescence microscope (Keyence, Biorevo).

To assess draxin–AP binding to neurites, RL explants were cultured for 72 h followed by draxin–AP and control–AP binding experiments, which were performed using a previously described protocol [19] and in the conditioned media described above.

### 2.5. Quantification of axon outgrowth and nucleokinesis

For bath culture experiments, the longest 40 neurites per explant were measured using Keyence BZ software. Average neurite length was determined for each explant. Cell migration from explants was assessed using Sholl analysis [28]. Briefly, 10 concentric circles of gradually increasing radius (spaced every 40 μm from the edge of the explants) were drawn on digitized pictures of explants. Cell migration from the explants was estimated by counting the number of concentric circles that the cells reached after incubation. For co-culture experiments, explants were subdivided into four equal quadrants. The quadrant closest to the aggregates was designated as proximal, and the opposite quadrant was labeled distal. Neurite length in these two quadrants was measured using Keyence BZ software and the proximal/distal (P/D) ratio was determined.

### 2.6. Statistical analysis

Three pairs of E18 wild type and *draxin*$^{-/-}$ embryos (littermates) were used for *in vivo* analysis. For the ION, FoxP2-positive neurons were counted in at least five sections per embryo. For the LRN and ECN, areas positive for *Barhl1* were measured in at least five sections per embryo. We calculated the mean and

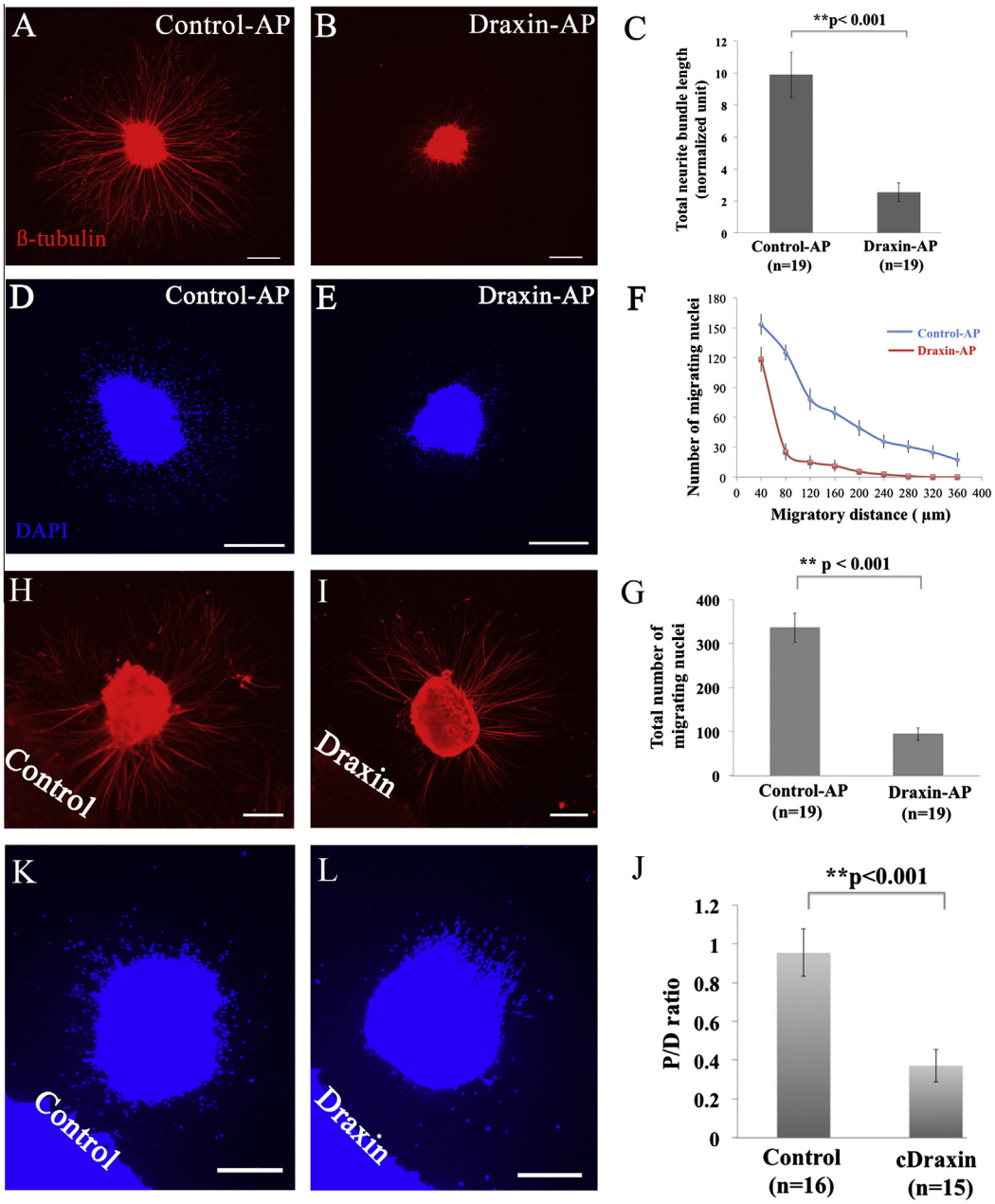


**Fig. 2.** Draxin inhibits ION axonal outgrowth and nuclei migration. E11.5 rhombic lip (RL) explants containing ION neurons were cultured in Matrigel for 72 h with either control–AP (A, D) or draxin–AP conditioned media (B and E). After culture, the explants were immunostained for ß-III tubulin (A and B) and the cell nuclei were visualized with DAPI (D and E). Compared to control–AP (A and C), neurite outgrowth was significantly inhibited by draxin-AP (**$p < 0.001$, B and C). Nuclei migration was significantly inhibited by draxin-AP (**$p < 0.001$, E and F) compared with control–AP (D and F). The total number of nuclei migrating from the explant was also reduced in the presence of draxin–AP (G). E11.5 RL explants were co-cultured in Matrigel with mock-transfected (H and K) and draxin-transfected 293 cell aggregates (I and L). In control samples, neurites emanating from the explants grew radially (H), while the growth of neurites in the proximal side of draxin expressing cell aggregates was markedly inhibited (I). The asymmetry in the neurite outgrowth in draxin-transfected 293 cell aggregates was indicated by the proximal/distal (P/D) ratio (**$p < 0.001$, Student's *t*-test, J). Radial nuclei migration was observed from the explant facing the mock-transfected 293 cell aggregates (K) whereas nuclei migration to the proximal area was inhibited in the explant facing the draxin-transfected 293 cell aggregates (L). Error bars indicate the mean ± SEM. Scale bars: A, B, D, E, 300 µm; H, I, K, L, 200 µm.

standard deviation (SD) for all data. Statistical analysis was performed using Student's *t*-test. $p < 0.05$ was considered statistically significant, $p < 0.001$ was considered highly significant (**$p$).

## 3. Results

### 3.1. Expression of draxin during the migration of PCN in mice

To determine the role of draxin during PCN development, we analyzed *draxin* expression in the developing hindbrain using *in situ* hybridization. PCN start to migrate from the RL at E11.5. At E13.5, ION neurons stop migrating and remain close to the floor plate, whereas LRN/ECN neurons migrate and cross the floor plate ventro-marginally to localize contralaterally to their origin. *Draxin* was highly expressed in the RL at E11.5 (Fig. 1A and B) and E13.5 (Fig. 1C). At E13.5, we also observed *draxin* expression in the LRN/ECN migratory stream (Fig. 1C), which was identified by positive expression of *Barhl1* (Fig. 1D). Thus, it appears that the expression pattern of *draxin* is of special interest for investigating cues that direct PCN migration.

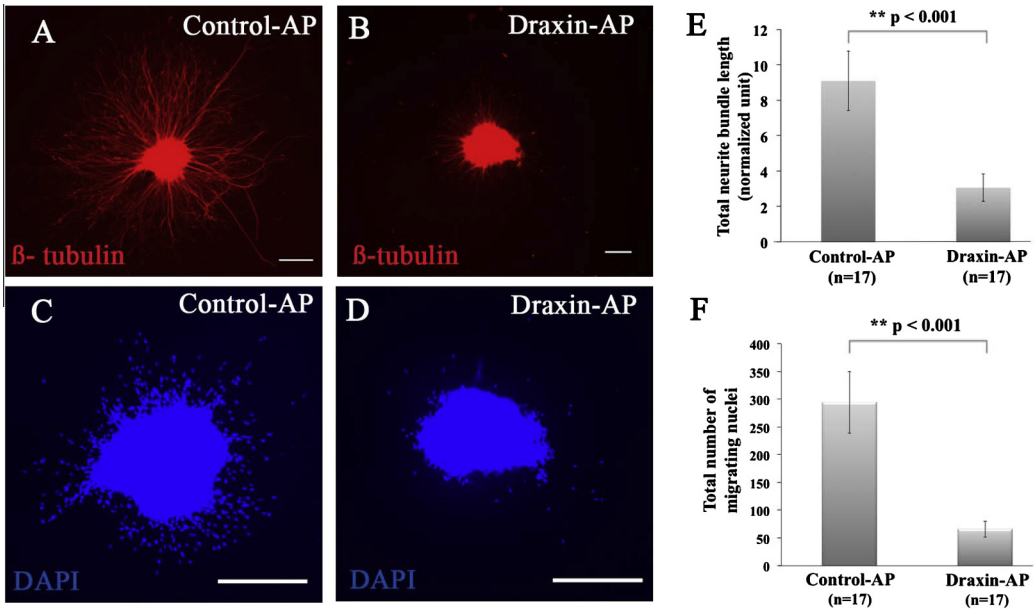


**Fig. 3.** Nuclei migration and axon outgrowth of LRN/ECN neurons is inhibited by draxin. E12.5 rhombic lip explants containing LRN/ECN neurons were cultured for 72 h either in the presence of control–AP (A, C) or draxin–AP conditioned media (B and D) and analyzed for axon outgrowth and nucleokinesis using ßIII-tubulin (A and B) and DAPI staining (C and D) respectively. Draxin–AP exerted inhibitory effects on neurite outgrowth (B and E) and on nuclear migration (D and F) compared with control-AP condition media (A, C, E, and F). Error bars indicate the mean ± SEM. Scale bars: in (A–D), 300 μm.

To investigate the distribution of draxin receptors, we performed draxin–AP binding assay on neurites derived from E11.5 RL explant cultures. We found strong draxin–AP binding in the neurites (Fig. 1E), compared with control–AP (Fig. 1F). This result suggests the presence of draxin receptor/s in PCN.

### 3.2. ION axon outgrowth and migration inhibited by draxin

*In vitro* culture experiments were carried out to assess the role of draxin in axon outgrowth and migration of ION neurons. RL explants from E11.5 embryos were used for this experiment, as they are an enriched source of ION neurons over other PCN at that stage [9,15]. RL explants were cultured in the presence of either control–AP or draxin–AP. Robust neurite outgrowth was observed from RL explants when cultured in control-AP conditioned medium (Fig. 2A and C), whereas neurite outgrowth was significantly reduced when explants were incubated with draxin–AP conditioned medium (Fig. 2B and C). To assess the migratory potential of ION neurons, we quantified cell movement away from the explants by applying a Sholl analysis [28]. We observed a significant reduction in the migration of ION from RL explants in the presence of draxin-AP compared to control-AP (Fig. 2D–F). Total number of nuclei migrating from the explant was significantly reduced in the presence of draxin-AP compared to control-AP (Fig. 2G).

We co-cultured RL explants from E11.5 wild type mouse embryos with 293 cell aggregates expressing draxin in Matrigel for 60 h. We used mock-transfected 293 cell aggregates as a control. We observed symmetrical neurite outgrowth from explants cultured with control-293 cell aggregates (Fig. 2H and J) whereas asymmetrical neurite outgrowth, mostly in the distal quadrant, was observed when confronted with 293-cells expressing draxin (Fig. 2I and J). This asymmetry is reflected by the P/D ratio (Fig. 2J). We also observed asymmetry in nuclei migration mainly in the distal quadrant when assessing cultures containing draxin-293 cell aggregates (Fig. 2K and L).

### 3.3. Draxin inhibits axon outgrowth and migration of LRN and ECN

To assess the influence of draxin on LRN and ECN neuronal migration, we used RL explants from E12.5 embryos; at E12.5, all ION neurons have left the lower RL. Thus, the majority of cells in these explants are LRN and ECN neurons [10]. We observed robust neurite outgrowth and nuclei migration when explants were cultured in the presence of control–AP (Fig. 3A, C, E). However, in the presence of draxin–AP, neurite outgrowth and nuclei migration were greatly inhibited (Fig. 3B, D, E). The total number of migrating nuclei was reduced in the presence of draxin–AP compared with control–AP (Fig. 3F).

### 3.4. PCN migration is unaltered in *draxin*$^{-/-}$ mice

To investigate whether PCN migration was defective in *draxin*$^{-/-}$ mice, we used E18 wild type and *draxin*$^{-/-}$ littermate embryos. To identify ION neurons, we used anti-FoxP2 antibody, which uniformly stained all ION neurons; ION markers such as Brn3.2 and Er81 cannot identify all ION neurons [17,22]. FoxP2 immunoreactivity was also observed in the ambiguus nucleus (AN) and dorsal nucleus of the twelfth nerve (N12n) [22].

We counted FoxP2-positive neurons in the floor plate and in the ION migratory stream of wild type (Fig. 4A, C, F) and *draxin*$^{-/-}$ (Fig. 4B, D, G) embryos. Although we observed a small reduction in the number of ION neurons in the floor plate of *draxin*$^{-/-}$ embryos (Fig. 4D) compared to wild type embryos (Fig. 4C), this difference was not statistically significant (Fig. 4E). However, in the ION migratory stream, we observed more FoxP2-positive cells in *draxin*$^{-/-}$ embryos (Fig. 4G and 4H) compared to wild type (Fig. 4F and H).

We also examined LRN and ECN neuronal distribution in *draxin*$^{-/-}$ embryos using *in situ* hybridization for *Barhl1* [23]. The position of the LRN and ECN neurons was unchanged in *draxin*$^{-/-}$ embryos compared to wild type and there were no significant differences in the distribution of ECN (Fig. 4I–K) and LRN (Fig. 4L–N) neurons between *draxin*$^{-/-}$ and wild type embryos (Fig. 4K and N).

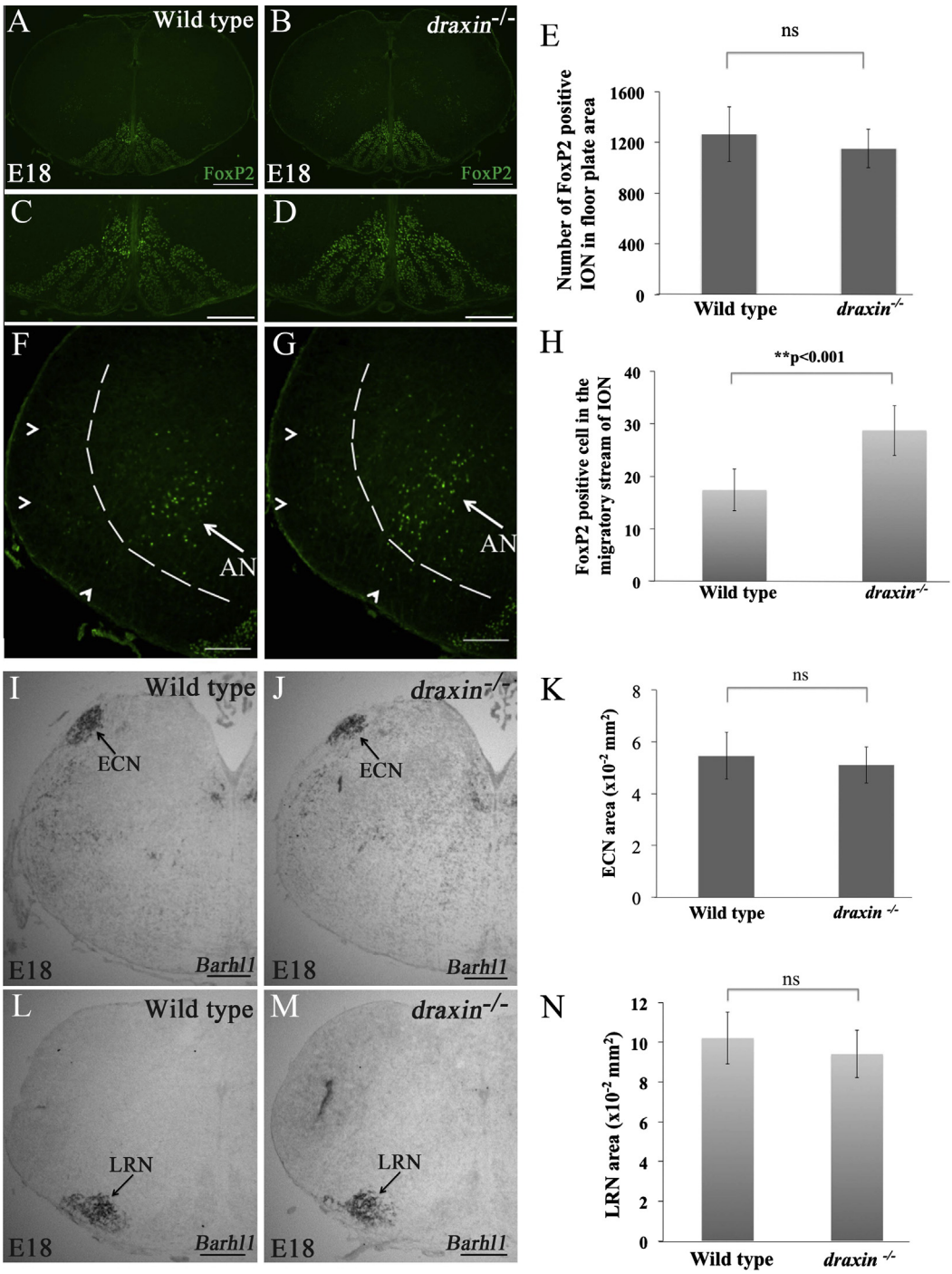


**Fig. 4.** Development of precerebellar nuclei in *draxin*$^{-/-}$ mice. Coronal sections of the inferior olive of E18 wild type (A, C, F) and *draxin*$^{-/-}$ (B, D, G) embryos were stained with anti-FoxP2 antibody. The total number of FoxP2-positive ION neurons in the floor plate was quantified; no significant difference was found between wild type (C and E) and *draxin*$^{-/-}$ embryos (D and E). Higher magnification images of the ION migratory stream (arrowheads) in wild type (F) and *draxin*$^{-/-}$ embryos (G) showed that more FoxP2-positive cells were observed in the ION migratory stream in *draxin*$^{-/-}$ embryos compared to wild type (H). FoxP2 immunoreactivity was also observed in the ambiguus nucleus (AN) (arrows, F and G). Coronal sections of the ECN from E18 wild type (I) and *draxin*$^{-/-}$ embryos (J) were hybridized with a *Barhl1* riboprobe to identify ECN cell bodies (arrow). LRN cell bodies were visualized using *Barhl1* expression in E18 wild type (L) and *draxin*$^{-/-}$ embryos (M). Both ECN and LRN size was reduced in *draxin*$^{-/-}$ embryos but no significant differences were found (K and N). Error bars indicate the mean ± SEM. Scale bars: A, B, 500 μm; F, G, 200 μm; C, D, I, J, L, M, 300 μm.

## 4. Discussion

Previous studies have demonstrated that various guidance molecules regulate the development of PCN. Several lines of evidence indicate that Netrin/DCC and Slit/Robo are the key modulators of PCN migration in the midline [12,14,16–18]. However, in knockout mice for these molecules, ION and LRN neurons still migrate away from the RL, suggesting that other repellents may be present in RL which initiates the migration towards floor plate [12,17].

Our expression data and culture experiments suggest that draxin is repulsive for axon outgrowth and nuclei migration of PCN subtypes. We also observed the presence of the draxin receptor in neurites derived from RL explants. Thus, draxin might directly regulate the development of these neurons *in vivo*. We observed

more FoxP2 positive cell in the migratory stream of ION neuron in *draxin*$^{-/-}$ compared with wild type embryo. However, we found no significant differences in overall distribution of ION, LRN and ECN neurons between *draxin*$^{-/-}$ and wild type embryos. One possible explanation for this is that draxin and other axon guidance cues may have redundant roles in PCN migration (Supplementary Fig. 1). For example, *Slit-1* and *Slit-2* double knockout mice show more significant PCN migration defects than single knockout mice, indicating multiple guidance cues working together [17]. From the expression pattern it is found that *Slit-2* expressed both in floor plate and roof plate and have repulsive role in migration of ION [9]. *Slit-1* is also expressed near the RL [9]. In addition, bone morphogenetic proteins (BMP) and growth/differentiation factors (GDF), which function as repellents for spinal cord commissural neurons [25–27], are also expressed in the RL. Thus, draxin may cooperate with other repulsive cues to regulate the development of PCN.

## Acknowledgments

We are very grateful to Dr. Giasuddin Ahmed and Athary Felemban for their generosity and cooperation. This work was supported by grants-in-aid from the Ministry of Education, Science, Sports and Culture of Japan to Y.S. and H.T.; and from the Takeda Science Foundation to Y.S.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.05.013.

## References

[1] C. Métin, R.B. Vallee, P. Rakic, P.G. Bhide, Modes and mishaps of neuronal migration in the mammalian brain, J. Neurosci. 28 (2008) 11746–11752.
[2] D. Kawauchi, H. Taniguchi, H. Watanabe, T. Saito, F. Murakami, Direct visualization of nucleogenesis by precerebellar neurons: involvement of ventricle-directed, radial fibre-associated migration, Development 133 (2006) 1113–1123.
[3] J. Altman, S.A. Bayer, Development of the precerebellar nuclei in the rat: II. The intramural olivary migratory stream and the neurogenetic organization of the inferior olive, J. Comp. Neurol. 257 (1987) 490–512.
[4] J. Altman, S.A. Bayer, Development of the precerebellar nuclei in the rat: IV. The anterior precerebellar extramural migratory stream and the nucleus reticularis tegmenti pontis and the basal pontine gray, J. Comp. Neurol. 257 (1987) 529–552.
[5] R.J. Wingate, The rhombic lip and early cerebellar development, Curr. Opin. Neurobiol. 11 (2001) 82–88.
[6] F. Bourrat, C. Sotelo, Migratory pathways and neuritic differentiation of inferior olivary neurons in the rat embryo. Axonal tracing study using the in vitro slab technique, Brain Res. 467 (1988) 19–37.
[7] F. Bourrat, C. Sotelo, Relationships between neuronal birthdates and cytoarchitecture in the rat inferior olivary complex, J. Comp. Neurol. 313 (1991) 509–521.
[8] J.D. Gilthorpe, E.-K. Papantoniou, A. Chédotal, A. Lumsden, R.J.T. Wingate, The migration of cerebellar rhombic lip derivatives, Development 129 (2002) 4719–4728.
[9] F. Causeret, F. Danne, F. Ezan, C. Sotelo, E. Bloch-Gallego, Slit antagonizes netrin-1 attractive effects during the migration of inferior olivary neurons, Dev. Biol. 246 (2002) 429–440.
[10] S. Alcantara, M. Ruiz, F. De Castro, E. Soriano, Netrin 1 acts as an attractive or as a repulsive cue for distinct migrating neurons during the development of the cerebellar system, Development 127 (2000) 1359–1372.
[11] I. de Diego, K. Kyriakopoulou, D. Karagogeos, M. Wassef, Multiple influences on the migration of precerebellar neurons in the caudal medulla, Development 129 (2002) 297–306.
[12] S. Marcos, S. Backer, F. Causeret, M. Tessier-Lavigne, E. Bloch-Gallego, Differential roles of netrin-1 and its receptor DCC in inferior olivary neuron migration, Mol. Cell Neurosci. 41 (2009) 429–439.
[13] H. Taniguchi, A. Tamada, T.E. Kennedy, F. Murakami, Crossing the ventral midline causes neurons to change their response to floor plate and alar plate attractive cues during transmedian migration, Dev. Biol. 249 (2002) 321–332.
[14] K.T. Yee, H.H. Simon, M. Tessier-Lavigne, D.M. O'Leary, Extension of long leading processes and neuronal migration in the mammalian brain directed by the chemoattractant netrin-1, Neuron 24 (1999) 607–622.
[15] F. Causeret, M. Hidalgo-Sanchez, P. Fort, S. Backer, M.-R. Popoff, C. Gauthier-Rouvière, et al., Distinct roles of Rac1/Cdc42 and Rho/Rock for axon outgrowth and nucleokinesis of precerebellar neurons toward netrin 1, Development 131 (2004) 2841–2852.
[16] E. Bloch-Gallego, F. Ezan, M. Tessier-Lavigne, C. Sotelo, Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons, J. Neurosci. 19 (1999) 4407–4420.
[17] T. Di Meglio, K.T. Nguyen-Ba-Charvet, M. Tessier-Lavigne, C. Sotelo, A. Chédotal, Molecular mechanisms controlling midline crossing by precerebellar neurons, J. Neurosci. 28 (2008) 6285–6294.
[18] V. Marillat, C. Sabatier, V. Failli, E. Matsunaga, C. Sotelo, M. Tessier-Lavigne, et al., The slit receptor Rig-1/Robo3 controls midline crossing by hindbrain precerebellar neurons and axons, Neuron 43 (2004) 69–79.
[19] S.M. Islam, Y. Shinmyo, T. Okafuji, Y. Su, I.B. Naser, G. Ahmed, et al., Draxin, a repulsive guidance protein for spinal cord and forebrain commissures, Science 323 (2009) 388–393.
[20] I.B. Naser, Y. Su, S.M. Islam, Y. Shinmyo, S. Zhang, G. Ahmed, et al., Analysis of a repulsive axon guidance molecule, draxin, on ventrally directed axon projection in chick early embryonic midbrain, Dev. Biol. 332 (2009) 351–359.
[21] G. Ahmed, Y. Shinmyo, I.B. Naser, M. Hossain, Olfactory bulb axonal outgrowth is inhibited by draxin, Biochem. Biophys. Res. Commun. 398 (2010) 730–734.
[22] M. Hashimoto, R. Ito, N. Kitamura, K. Namba, Y. Hisano, Epha4 controls the midline crossing and contralateral axonal projections of inferior olive neurons, J. Comp. Neurol. 520 (2012) 1702–1720.
[23] S. Li, F. Qiu, A. Xu, S.M. Price, M. Xiang, Barhl1 regulates migration and survival of cerebellar granule cells by controlling expression of the neurotrophin-3 gene, J. Neurosci. 24 (2004) 3104–3114.
[24] K. Ohta, D. Tannahill, K. Yoshida, A.R. Johnson, G.M. Cook, R.J. Keynes, Embryonic lens repels retinal ganglion cell axons, Dev. Biol. 211 (1999) 124–132.
[25] A. Augsburger, A. Schuchardt, S. Hoskins, J. Dodd, S. Butler, BMPs as mediators of roof plate repulsion of commissural neurons, Neuron 24 (1999) 127–141.
[26] J. Alder, K.J. Lee, T.M. Jessell, M.E. Hatten, Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells, Nat. Neurosci. 2 (1999) 535–540.
[27] S.J. Butler, J. Dodd, A role for BMP heterodimers in roof plate-mediated repulsion of commissural axons, Neuron 38 (2003) 389–401.
[28] D.A. Sholl, Dendritic organization in the neurons of the visual and motor cortices of the cat, J. Anat. 87 (1953) 387–406.