Synaptic Input as a Directional Cue for Migrating Interneuron Precursors

*Running title: Synapses guide neuronal migration*

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Summary statement:

Precursors of cerebellar inhibitory interneurons receive an unprecedented early synaptic input while they are still migrating, and this input directs the mobility and directionality of these cells.

**ABSTRACT**

During CNS development, interneuron precursors have to migrate extensively before they integrate in specific microcircuits. Known regulators of neuronal motility include classical neurotransmitters, yet the mechanisms that assure interneuron dispersal and interneuron / projection neuron matching during histogenesis remain largely elusive. We combined time-lapse video-microscopy and electrophysiological analysis of the nascent cerebellum of transgenic Pax2-EGFP mice to address this issue. We found that cerebellar interneuronal precursors regularly show spontaneous postsynaptic currents, indicative of synaptic innervation, well before settling in the molecular layer. In keeping with the sensitivity of these cells to neurotransmitters, ablation of synaptic communication by blocking vesicular release in acute slices of developing cerebella slows migration. Significantly, abrogation of exocytosis primarily impedes the directional persistence of migratory interneuronal precursors. These results establish an unprecedented function of early synaptic innervation of migrating neuronal precursors and demonstrate a role for synapses in the regulation of migration and pathfinding.

**INTRODUCTION**

During development of the vertebrate nervous system, neuronal precursors migrate extensively before they reach their adult positions and terminally differentiate (Valiente and Marín, 2010). Both in the developing forebrain and cerebellum, projection neurons destined to form cortical structures originate in the ventricular epithelium, from where they follow a radial route to reach their adult positions. Precursors of inhibitory interneurons, on the other hand, follow more varied routes to reach their target positions, which generally include extensive tangential relocations (Goldowitz and Hamre, 1998; Letinic et al., 2002; Tan et al., 1998). The eventual establishment of efficiently wired central nervous circuits is critically dependent on a precise coordination of these morphogenetic migrations (Marín et al., 2010). In fact, an increasing number of neurologic and psychiatric conditions is being associated with neuronal migration deficits (Barkovich et al., 2012; Peñagarikano et al., 2011; Rivière et al., 2012).

In contrast to signals and mechanisms subserving cortical projection neuron migration (Jossin and Cooper, 2011; Rice and Curran, 2001), our mechanistic understanding of interneuronal pathfinding, dispersal and eventual matching with projection neurons is still rather fragmentary (Marín et al., 2010). Cortical inhibitory interneurons are either generated late in ontogenesis or have to migrate for extended periods to their final positions (Leto et al., 2009; Rymar and Sadikot, 2007). Consequently, they have to navigate the nascent lattice formed by earlier settled projection neurons to reach their specific target areas. A key question is whether and how the latter may direct interneuron migration and maturation to assure generation of proper functioning CNS microcircuits. Intriguingly, an increasing body of evidence indicates that mobility of neuronal precursors, including immature interneurons, is sensitive to neurotransmitters (Bortone and Polleux, 2009; de Lima et al., 2009; Komuro and Rakic, 1993; Manent and Represa, 2007).

Here, we follow up on this lead and ask how neural communication might affect corticogenesis, and in particular directed interneuron migration in the cerebellar cortex. This cortical structure seems uniquely suited to address such a general question: Compared to the forebrain cortex, the cerebellar cortex is evolutionary rather conserved (Bell et al., 2008). It is constituted by a limited number of genetically well characterized cell types with distinct developmental histories (Goldowitz and Hamre, 1998; Schilling et al., 2008). Its inhibitory interneurons, and particularly those in the molecular layer (ML), arguably form one of the largest and best characterized groups of inhibitory interneurons in the vertebrate central nervous system (see, e.g. Markram et al., 2004; Kepecs and Fishell, 2014; Carter and Regehr, 2002; Jorntell et al., 2010; Kim et al., 2014).

Among the first cerebellar neurons formed are Purkinje cells, inhibitory interneurons of the deep nuclei, and inhibitory interneurons of the future granule cell layer (GL). In the mouse, these go through their last mitosis from around embryonic days 10.5-13.5, 10.5-11.5, and 13.5 to postnatal day 1, respectively (e.g. Sudarov et al., 2011; for a recent review and further references, see Leto et al., 2016), and subsequently migrate radially to form the cerebellar anlage. From about embryonic day 12.5-16, Math1-positive cells migrate from the rhombic lip (Ben-Arie et al., 1997) underneath the pial surface of the cerebellar anlage to form the external granule cell layer (EGL), where they proliferate extensively up to about postnatal day 15. Math1-positive cells give rise to all excitatory cerebellar neurons, including granule cells, unipolar brush cells and excitatory cells in the deep nuclei (Machold and Fishell, 2005). Both afferent climbing fibers (Kita et al., 2015; Wassef et al., 1992) and mossy fibers reach the cerebellar anlage before or around birth (Ashwell and Zhang, 1992; Grishkat and Eisenman, 1995; Nunes and Sotelo, 1985).

Lastly, inhibitory interneurons resident in the molecular layer (ML) are formed over a rather protracted period, from about embryonic day 15 to postnatal day 7 (Leto et al., 2009; Sudarov et al., 2011). They have to traverse the nascent white matter (WM), the emerging internal granule cell layer (IGL), and eventually the Purkinje cell layer (PCL) to reach the molecular layer. There, they initially accumulate at the border of the EGL before they take up their adult positions (Leto et al., 2009; Weisheit et al., 2006; Zhang and Goldman, 1996; Simat et al 2007; Maricich and Herrup, 1999). According to current thought, it is only then that they become synaptically integrated with granule cell afferents and Purkinje neurons, the sole projection neurons of the cerebellar cortex (Schilling, 2013).

In striking contrast to this detailed descriptive understanding, the mechanisms that direct cerebellar interneuron migration and dispersion remain elusive. To address this issue, we combined two-photon time-lapse imaging and patch-clamp recordings to characterize genetically tagged, migrating precursors of cerebellar inhibitory interneurons. Unexpectedly, we found that these precursors in transit are synaptically innervated. Elimination of synaptic exocytosis reduced the speed and, conspicuously, the directionality of migration. These findings establish a hitherto unknown, early synaptic innervation of developing interneurons. Further, they document that such synapses implement a mechanism that allows these cells to properly navigate the nascent cerebellar cortex.

**RESULTS**

**Basic morphological and electrophysiological characteristics of migrating interneuron precursors**

In acute cerebellar slices of 7–9 day old mice expressing EGFP from the locus of the paired-box gene, *Pax2* (Pfeffer et al., 2002; Weisheit et al., 2006), precursors of ML inhibitory interneurons can be recognized as strongly EGFP-positive, slender, fusiform cells. They may be found within the prospective WM, the IGL, and the nascent ML, through which they consecutively migrate to reach their adult position. Their morphology and mobility towards the developing ML (Fig. 1,2; see also Movies 1-3) allow to readily distinguish them from the larger, roundish, and already settled inhibitory interneurons of the IGL ("Golgi cells"), which also express Pax2 (Maricich and Herrup, 1999). Within the ML, newly arrived Pax2-EGFP cells could be readily distinguished from more mature ML interneurons by their strong EGFP signal, their preferential orientation perpendicular to the Purkinje cell layer, and their mobility. After entering the ML, these cells first translocate towards the border of the ML and the EGL (Simat et al., 2007; Weisheit et al., 2006). Upon their subsequent embedding in the growing ML, they rapidly down-regulate Pax2 and Pax2-EGFP (Glassmann et al., 2009; Weisheit et al., 2006), and they can be recognized as dimly stained, immobile cells with their long axis preferentially parallel to the Purkinje cell layer (Fig. 2B).

Strongly Pax2-EGFP positive, immature precursors of ML inhibitory interneurons (henceforth referred to as Pax2-cells) were characterized by a high input resistance (Rin > 1 GΩ in all but 6 / 176 cells) and a capacitance of 9.4 ± 0.4 pF (mean ± SEM). Their whole-cell current pattern (Fig. 3A) was made up by transient and delayed rectifier K+ currents and voltage-gated, TTX-sensitive Na+ currents (Fig. S1). We did not observe spontaneous action potentials in these cells. Upon current injection (Fig. 3B), Pax2-cells in the prospective WM (*n* = 7), the nascent IGL (*n* = 8), and the ML (*n* = 10) generated spikelets, but no full action potentials. Their Na+ current density was 0.7 ± 0.1 pA/µm2.

This clearly distinguishes Pax2-cells from Pax2-EGFP positive inhibitory interneurons of the granule cell layer, which are characterized by a much larger membrane capacitance (47.8 ± 5.3 pF, n = 13). This latter value is in excellent agreement with data reported previously (Dieudonné, 1995; Dieudonné, 1998; Elisabetta Cesana et al., 2006). The electrophysiological properties of Pax2-cells are also clearly distinct from those of mature inhibitory ML interneurons, which show spontaneous action potentials of up to 35 Hz and Na+ current densities beyond 30 pA/µm2 (Ruigrok et al., 2011; Southan and Robertson, 1998).

Application of GABA (100 µM) or the GABAA receptor agonist muscimol (300 µM) to Pax2-cells held at −70 mV consistently resulted in inward currents (0.03 ± 0.01 pA/µm2; *n* = 14 and 0.1 ± 0.01 pA/µm2; *n* = 13, respectively) that were blocked by bicuculline (100 µM) by about 85% (to 0.001 ± 0.001 pA/µm2; *n*= 4 and 0.02 ± 0.01 pA/µm2; *n*= 4 for GABA and muscimol, respectively; Fig. 3C). The reversal potential at the maximal slope conductance was ‑0 ± 2 mV (*n* = 12), confirming a Cl--mediated current (Fig. S2A–A’’).

Further, these Pax2-cells in transit also express ionotropic receptors of the AMPA/kainate (KA)-type (Fig. 3D and Fig. S2B-B’’). We noted that Pax2-cells migrating through the WM display significantly smaller KA (300 µM)-induced receptor currents (0.3 ± 0.06 pA/µm2; *n*= 14; Vh= −70 mV) than those in the ML (0.95 ± 0.13 pA; *n*= 14; p < 0.001 for WM vs. ML) indicating developmental changes of receptor density or gating properties (Smith et al., 2000), e.g. due to a switch in flip/flop splicing (Monyer et al., 1991). Cyclothiazide (100 µM), an allosteric modulator of the flip versions of AMPA receptors (Partin et al., 1993) potentiated KA-induced responses in Pax2-cells in the WM (by 86 ± 13% at –70 mV, *n*= 12, p < 0.01) but not in the ML (15 ± 6%, *n* = 9, p = 0.3). NBQX (20 µM) completely blocked KA-induced currents (*n* = 8). Our data are consistent with the reported relative increase of AMPA-receptor flop variants (Monyer et al., 1991) and/or increased hetero-oligomerization (Liu and Cull-Candy, 2002) with ongoing development.

**Migrating precursors receive synaptic input**

Unexpectedly, we observed spontaneous postsynaptic currents (sPSCs) in Pax2-cells, suggesting that these immature cells in transit receive synaptic input (Fig. 3E–G). sPSCs could be recorded from 15 out of 17 Pax2-cells in the ML and 5 / 6 cells transiting the nascent IGL. We could not detect sPSCs in Pax2-cells transiting the prospective WM (*n*= 11).

Next we wanted to confirm that Pax2-cells receive synaptic input while actually migrating. Over a period of several hours these cells travel impressive distances and may traverse the entire cerebellar cortex (Movie 1). We used high-resolution time-lapse video-microscopy and selected cells that had moved for at least 3 µm (*n* = 35; median 11.2 µm, range 3–43 µm) for subsequent patch-clamp recording. Wash-out of EGFP during recording confirmed that the visually selected cell was impaled by the patch pipette.

Consistent with the above data for cells selected on their position and morphology alone, Pax2-cells that had migrated immediately before patch-clamping received synaptic input, depending on their position. Eight out of 13 cells in the IGL, 15/19 in the ML, but 0/3 cells in the prospective WM showed sPSCs. The synaptic origin of this activity in Pax2-cells traversing the IGL and ML was further supported by the increased rate of miniature PSCs (mPSCs) following application of the Ca2+ ionophore ionomycin (3 µM; Fig. 3L–N). This agent increases the intracellular Ca2+ concentration and thus enhances release from the readily releasable vesicular pool in synaptic terminals. In the WM, in the absence of TTX, no sPSCs were observed in Pax2-cells, even after application of ionomycin.

Analysis of current kinetics distinguished two types of sPSCs with decay time constants of < 1 ms and about 12 ms, respectively (Fig. 3E,F,H,I,K upper panel; and Fig. 3E,G,K lower panel). Further analysis showed that the former were sensitive to NBQX and APV (i.e., were mediated by ionotropic glutamate receptors; Fig. 3J), whereas the latter were abolished by bicuculline (i.e., mediated by GABAA receptors; Fig. 3H). As expected for AMPA/KA and GABAA receptors, the reversal potentials of the fast and slow sPSCs were 0 and – 40 mV (Fig. S3). The rise times of these sPSCs were 0.37 ± 0.01 ms for fast decaying sPSCs (*n* = 1843, 20 cells) and 0.46 ± 0.01 ms for slowly decaying sPSCs (*n* = 384, 17 cells), respectively.

All cells with sPSCs (*n*= 43) received glutamatergic input while GABAergic input was less abundant (*n*= 35/43). There was no indication of layer-specific differences in GABAergic or glutamatergic innervation.

On their way into the ML, Pax2-cells migrate subsequently through the WM, the nascent IGL, the PCL, and in the ML up to the EGL (for an overview, see Fig. 4A). To assess the density of presynaptic elements that may contact Pax2-cells en route, we quantified synaptophysin-immunoreactive puncta in these layers. In line with the layer specific frequency of sPSCs, the density of synaptophysin-positive, putative presynaptic elements increased from the WM to the ML (Fig. 4B–D). Ultrastructural analysis of Pax2-cells in the lower ML confirmed the occurrence of synaptic structures on these cells (Figs 5,S4). This particular location was chosen since expression of Pax2-EGFP allowed to select reliably interneuron precursors migrating towards more superficial positions over those that have already settled in the ML (Weisheit et al., 2006). This ultrastructural evidence together with the fast rise times of the sPSCs suggests that the latter originate from direct synaptic innervation rather than from transmitter spill-over.

**Synaptic innervation and directional persistence of migrating Pax2-cells**

To probe the functional significance of this surprising and hitherto unknown synaptic input, we used tetanus toxin (TeNT) and Co2+ to abrogate pre-synaptic vesicular release. Of these agents, TeNT is more efficient as it inhibits exocytosis by cleaving synaptobrevin. We verified the efficacy of these agents in our preparation by monitoring their effect on the frequency and amplitudes of sPSCs recorded from Purkinje neurons. These cells, rather than sparsely innervated Pax2-cells, were chosen because their already strong innervation allows a dependable assessment of synaptic activity.Taking cell-wise sPSC average-amplitudes multiplied with numbers of events occurring within 5 min as a proxy of synaptic innervation, we observed that TeNT eliminated 99.7 + 0.2% (mean + SEM) of all synaptic input. Co2+ reduced synaptic input by 80.1 + 3.7 % (5 cells, from 5 slices from 2 animals, measured before and after Co2+ wash-in). Note that remaining sPSCs under Co2+ represent essentially spontaneous, action potential independent release (“miniature PSCs”).

We analysed the effects of TeNT on cell motility in three slices obtained from three animals. Migration of 917 Pax2-cells was monitored prior to treatment (exemplary slice shown in Movie 2) and, within the very same volume elements, another 651 cells were monitored following 120 min incubation with TeNT (100 µg/ml). TeNT resulted in robust reductions of Pax2-cell displacement, (average) speed and, intriguingly, the ratio of displacement and total path length, which provides a measure of directionality (Batschelet, 1981; Benhamou, 2004) (Figs 6; S5 for methodological details). Sham incubation (without TeNT) did not bring about such changes (Figs 6, S5D). Exemplary tracks of control cells and cells after incubation with TeNT are documented in Movie 3.

Mechanistically, the nonspecific antagonist of voltage-gated Ca2+ channels, Co2+ (used at 2 mM; 20 min wash-in; three slices from three animals) prevents secretion upstream of the site of action of TeNT. It also allows avoiding the protracted incubation needed with TeNT. Further, potential nonspecific side effects of these two agents may be predicted to be distinct. Intriguingly, the effects of Co2+-reduced synaptic release on cell motility and directionality were virtually identical to those observed following TeNT blockade of exocytosis (Fig. 6).

To probe the loss of directionality following abrogation of pre-synaptic vesicular release directly and at the high temporal resolution afforded by our sampling rate, we analysed turning angles formed by the track-segments ("steps") joining cell positions recorded at subsequent time points, which were separated by ~ 42 seconds (see Fig. S6 for a scheme). These angles provide a measure for directional persistence: for cells with high directional persistence, they are centred about zero degrees. In contrast, tracks of cells with low directional persistence are characterized by highly variable inter-step angles. Under control conditions (Fig. 7A‑C), angles between subsequent steps (Δφ) were concentrated about 0 in the coronal and sagittal planes, indicating preferentially directional translocation. Following incubation with TeNT (Fig. 7A’,A’’) or wash-in of Co2+ (Fig. 7C’,C’’), the frequency of Δφ centred about 0 was conspicuously reduced, and larger Δφ values occurred more frequently (Fig. 7A’,C’). This indicates that following synaptic silencing, directional persistence of migrating Pax2-cells was reduced. To wit, this should not be confused with an overall loss of directed migration. However, the loss of directional persistence makes directed migration much less efficient.

Another way to measure directional persistence is by quantifying the turning angles without reference to anatomical planes (Fig. S6, right panel). This reduces the dimensionality of the data to one and conveniently allows their statistical analysis with Kuiper’s test. This confirmed the visual impression conveyed by the density plots that TeNT (Fig. 7A’’) and Co2+ (Fig. 7C’’) had significant effects on directional persistence (p < 0.001) whereas sham incubation (Fig. 7B’’) had not (p = 0.221).

An intriguing question here is whether the effects of synaptic activity on Pax2-cell motility are related to the localized nature of synaptic signalling. Conversely, one might invoke the contribution of synaptic activity to ambient transmitter concentrations to explain the effects observed. To address this question, we blocked ionotropic and metabotropic receptors of glutamate and GABA, but also purines (P2R) and several cytokines and for neurotrophins (TrkA/B/C, p75; see Materials and Methods for an overview of the receptors targeted by the blockers used), which have previously been shown to impinge on neuronal translocation. In contrast to elimination of synaptic vesicular release by TeNT or Co2+, global elimination of transmitter action by receptor blockade resulted in at best minor and highly variable effects on all motility parameters assessed (Fig. 6).

**DISCUSSION**

Together, these morphological and functional findings document that precursors of ML interneurons in transit through the cerebellar cortex receive GABAergic and glutamatergic synaptic input, and that synaptic input modulates their migratory behaviour. To the best of our knowledge, synapses have so far not been described on migratory neuronal precursors.

The fast rise times of the post-synaptic currents observed in Pax2-cells are also compatible with ectopic release, i.e. release from pre-synaptic elements in close contact with cells that lack typical postsynaptic morphology (Matsui and Jahr, 2004). Yet they rule out spill-over transmission. We prefer the term synaptic, as our ultrastructural data document the existence of morphologically bona fide synapses on Pax2-cells. We note that ectopic release, should it be involved, would still constitute a highly localized signal, functionally equivalent to synapses (cf also the discussion in Matsui and Jahr, 2004).

While it is well established that neurotransmitters or blockade of their receptors affect neuronal migration (Bolteus and Bordey, 2004; Bortone and Polleux, 2009; Cuzon et al., 2006; de Lima et al., 2009; Komuro and Rakic, 1993; Manent and Represa, 2007; Manent et al., 2005), and notably speed, the present data also unveil a hitherto unknown effect of neurotransmitter signalling on the directionality of migration. This effect was specifically observed following ablation of presynaptic release. Thus, pre-synaptic elements may function as activity-modulated guideposts for migrating interneuronal precursors that navigate the nascent cerebellar cortex. This conclusion is underscored by the outstanding specificity of tetanus toxin for targeting synaptic release. In particular, this agent does not interfere with neurite outgrowth (Verderio et al., 1999), which might interfere with migration, and which has been observed when (synaptic) release was eliminated by ablation of Munc 18-1 and Munc 13 (Broeke et al., 2010).

At first sight, the distinct effects of ablation of vesicular release and receptor blockade may seem counter-intuitive. Yet if one contrasts how TeNT (or Co2+) and receptor blockade act on transmitter-mediated signals perceived by migrating cells (schematic Fig. 8), these differences provide a first clue how the hitherto unknown synaptic input to migrating neurons may mechanistically control their directionality and locomotion.

Traditionally, effects of transmitters on the motility of neuronal precursors have been surmised to be exclusively due to ambient, paracrine acting transmitters (Bolteus and Bordey, 2004; Bortone and Polleux, 2009; Cuzon et al., 2006; de Lima et al., 2009; Komuro and Rakic, 1993; Manent and Represa, 2007; Manent et al., 2005), i.e. a non-localized, or isotropic, signal. In contrast, synaptic input as identified here, results in highly anisotropic signals. Notably, synaptic input onto Pax2-cells does not generate action potentials and thus results in a localized, rather than a propagating signal. This conclusion may be reached considering that no action potentials, not even spikelets, could be elicited from Pax2-cells by current injections up to 40 pA (see first 5 sweeps in Fig. 3B; counting from bottom to top), i.e. well beyond the amplitudes of sPSCs recorded from these cells.

Suppression of synaptic release abrogates this anisotropic input to migrating cells. It will eventually also dampen global, isotropic (paracrine) input due to its effect on ambient transmitter concentrations. Yet as the latter are likely regulated, in the developing brain, primarily by non-synaptic mechanisms (Luhmann et al., 2015; Manent et al., 2005), e.g. transmitter-permeant anion channels (e.g. Liu et al., 2009), neurotransmitter transporters (possibly working in reverse; cf Raiteri and Raiteri, 2015), or insufficient transmitter uptake (by glial cells; e.g. Komuro and Rakic, 1993) synaptic silencing will not effectively shut down isotropic transmitter effects (Fig. 8 middle). In contrast, receptor antagonists eliminate signalling completely and irrespective of its source (Fig. 8 right).

Thus, the distinct effects of blockade of vesicular release and receptors may be rationalized by a model that combines a permissive global (isotropic, paracrine) input with a local (anisotropic, synaptic) instructive signal to regulate neuronal migration and pathfinding. The permissive signal is required to keep the cells' locomotor apparatus malleable and capable to react to a localized (guiding) signal. In the absence of localized signals only (i.e. following incubation with TeNT or Co2+), cell movements become erratic and disoriented. In the absence of all external signals (i.e., following receptor blockade), cell locomotion initially persists due to the internal inertia of its locomotor system: Eventually, though, its motility will also deteriorate, as the decay of its locomotor system is no longer balanced by reorganization dependent on external input.

This model is parsimonious and explains all of the observed effects without the need to invoke some enigmatic, synaptically released agent directing Pax2-cell migration. It provides a consistent and natural explanation for both, the relatively high variability of the effects seen following receptor blockade, and the observation that receptor blockade does eventually change cell mobility, when observed over more protracted time periods (but also at much sparser sampling rates) as we did here (e.g. Bolteus and Bordey, 2004; Bortone and Polleux, 2009; Cameron et al., 2009; Cuzon et al., 2006; de Lima et al., 2009; Komuro and Rakic, 1993; Manent and Represa, 2007; Manent et al., 2005). Further, it may be noted that the model proposed is conceptually equivalent to the well-established dependence of axonal guidance signalling, where the attractive or repulsive interpretation of one signal may be switched by a second, permissive signal (or internal state) of the receptive cell (see Naoki et al., 2016 and further references therein). Third, this model may also be seen as an instance of a more general model in which contrasting local and global effects are integrated to allow cells to steer in shallow gradients and to avoid adaption (Hecht et al., 2011; Xiong et al., 2010). These conceptual parallels may also help to conceive future research to further identify the molecular mechanisms underpinning the observed migratory behaviour.

To date, the mechanisms which direct the fate, differentiation and spatial dispersal of cerebellar inhibitory interneurons remain elusive. Recent experimental evidence (Leto et al., 2009) revealed that post-mitotic precursors of these cells retain a considerable degree of plasticity while in transit from the ventricular zone to their adult positions, and are sensitive to, and dependent on, instructive signals received en route. The present results document that migration of inhibitory interneuron precursors is sensitive to pre-synaptic vesicular release; more significantly, we show that early synaptic activity provides a directional cue for these cells.

This suggests a new function for released neurotransmitters beyond those established for neural precursor proliferation (e.g.Liu et al., 2005; LoTurco et al., 1995), motility (e.g.Bouzigues et al., 2007; Manent et al., 2005) and network integration (e.g.Ge et al., 2006; for a review seeSpitzer, 2006). We demonstrate that transmitters act on developing cells in transit in a highly localized manner, indicating a role for synaptic innervation in early neural histogenesis.

Abrogation of synaptic release onto Pax2-cells reduced their displacement by some 40 % (Fig. 6). This sets a frame for effects to be attributed to synaptic activity for the migration and dispersal of basket/stellate cells *in vivo*. Further, the spatio-temporal resolution of the present data allowed us to identify changes in cellular directionality, rather than speed, as the major determinant of displacement. As already alluded to above, the robust effect of synaptic input on directionality, resp. directional persistence described here for Pax2-cells is in keeping with models (Hecht et al., 2011; Xiong et al., 2010) that explain how localized input enables cells to navigate molecular gradients generated by paracrine release and to avoid adaptation.

Migrating Pax2-cells analysed presently (i.e., in the p7–9 cerebellar anlage) will eventually settle in the upper part of the molecular layer (Leto et al., 2009; Leto et al., 2011) to which their dendrites will also remain confined (e.g. Rakic, 1972; Sultan and Bower, 1998). There, they will form excitatory synapses with granule cell-derived parallel fibres and inhibitory synapses with each other (for a review and detailed references on the synaptic wiring of ML interneurons, see Schilling, 2013).

Potential candidates providing afferent input to Pax2-cells en route include inhibitory interneurons of the granule cell layer and parallel fibres in the lower ML. The latter will not synapse on Pax2-cells analysed here once they have reached the upper ML. However, we consider parallel fibres an unlikely source of developmental synaptic input to migrating Pax2-cells, given that there is no experimental evidence supporting spontaneous or triggered granule cell activity during the critical developmental period analysed here (for an extensive recent review, see Sassoe-Pognetto and Patrizi, 2013).

GABAergic Purkinje cells, glutamatergic mossy and/or climbing fibres or developmental, intermediate forms of the latter two (Mason and Gregory, 1984) seem more likely candidates to provide (transient) afferents to mobile Pax2-cells. First, they are known to be active during the early postnatal period of cerebellar development (e.g. Puro and Woodward, 1977; Shimono et al., 1976; Sokoloff et al., 2015). Second, at least mossy fibres and climbing fibres, are notorious for establishing developmentally transient synapses (e.g. Kalinovsky et al., 2011; Mason and Gregory, 1984; Watanabe and Kano, 2011).

The finding that Pax2-positive precursors of (upper) ML interneurons are synaptically innervated in areas not targeted by the afferents of their adult forms implicates that they experience a switch in synaptic input as they migrate to their final destinations. This switch would qualitatively differ from the well-known developmental synaptic remodelling occurring on more mature post-migratory cells to assure adequate matching of pre- and post-synapses (Gianola et al., 2003; Kalinovsky et al., 2011; Watanabe and Kano, 2011; Watt et al., 2009).

As the reduced preparation used here preserves only intra-cerebellar sources of mossy fibres, i.e. those originating in deep nuclei (Batini et al., 1992), the impact of the mossy fibre system on Pax2-cell migration has probably been underestimated.

Our results identify a novel mechanism that allows synaptic activity in long-distance projections to fine-tune local circuit formation, by impinging on interneuron positioning during development.

**MATERIALS AND METHODS**

**Animals**

Mice expressing EGFP from the *Pax2* locus (Pax2-EGFP; line BAC#30 Pfeffer et al., 2002; Weisheit et al., 2006) were kept as heterozygotes on a C57BL/6 background. All animal handling was done in accordance with local governmental and institutional animal-care regulations.

**Preparation of cerebellar slices**

Mice of either sex (p7–9) were anaesthetised and decapitated. Parasagittal slices (200 or 300 µm) were cut from the cerebellar vermis using a vibratome (VT 1000 S or 1200 S, Leica) and collected in ice-cold buffer containing (in mM): 87 NaCl, 2.5 KCl, 1.25 NaH2PO4, 7 MgCl2, 0.5 CaCl2, 25 NaHCO3, 25 glucose, 75 sucrose (347 mOsm) gassed with carbogen. They were stored for 30 min at 32 °C, cooled to room temperature (RT; ~24 °C) over a period of 30 min, and transferred to artificial cerebrospinal fluid (ACSF, RT) containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 2 CaCl2, 26 NaHCO3 and 10 glucose.

**Patch-clamp recordings**

Slices were viewed with infrared DIC-optics. Pax2-cells in lobule IV were identified by their expression of EGFP (using epifluorescence; Axioskop FS2, Zeiss, or DM 6000 CES, Leica microscopes) and their distinct morphology. Whereas stationary interneurons of the granule cell layer, which also express Pax2, are roundish with a diameter of about 12–15 µm, Pax2-cells impressed as slender cells with a perikaryon measuring some 12 × 5 µm. They were typically oriented perpendicular relative to the Purkinje cell layer while in transit through the cerebellar cortex. Lastly, we note that mature interneurons of the granule cell layer, including Golgi cells, showed a characteristic electrophysiological signature and were able to generate action potentials which unequivocally distinguished them from Pax2-cells. Cells were recorded in the whole-cell mode. During recording, slices were continuously perfused with ACSF, if not stated otherwise. Patch pipettes (borosilicate capillaries) had resistances of 3–4.5 MΩ and were filled with (in mM): 130 KCl, 3 Na2-ATP, 2 MgCl2, 0.5 CaCl2, 5 BAPTA, 10 HEPES; or 130 CsCl, 3 Na2-ATP, 2 MgCl2, 0.5 CaCl2, 5 BAPTA, 10 HEPES; or 125 K-gluconate, 2 Na2-ATP, 2 MgCl2, 0.5 EGTA, 10 HEPES, 20 KCl, 3 NaCl; or 125 Cs-gluconate, 2 Na2-ATP, 2 MgCl2, 0.5 EGTA, 10 HEPES, 20 CsCl, 3 NaCl (all solutions pH 7.25–7.3; liquid junction potentials corrected for gluconate solutions). EGFP-washout was used to confirm that recordings were actually obtained from Pax2-cells. To allow visualization of cells after EGFP-washout, internal solutions also contained 0.05% dextran-conjugated TRITC or 0.1% dextran-conjugated Texas Red (both MW 3000, Molecular Probes). Data were recorded with EPC 8, 9 or 800 amplifiers, filtered at 1–3 kHz, sampled at 0.1–30 kHz, and digitized with DAAD converters (ITC 16 or LIH 1600) controlled by TIDA software (HEKA Elektronik). Test pulses were elicited between −160 and +70 mV (before and after adding TTX, 1 µM), subsequent to applying conditioning pre-pulses (to −110 and −10 mV) to remove current inactivation. AMPA/KA- and GABAA-receptors were analysed in solutions containing bicuculline (10 µM) or picrotoxin (50 µM) and NBQX (10–20 µM) or CNQX (10 µM), respectively, and 10 mM BaCl2, 4 mM 4-AP, 30 µM CdCl2, 1 µM TTX, 50 µM DL-APV or 20‑25 µM D-APV, 135 mM NaCl, 5 mM KCl, 2 mM MgCl2, 2 mM CaCl2, 10 mM glucose, 10 mM HEPES (Figs 3C,D,S2). Agonists were bath-applied. Data were analysed with Igor Pro 6 (WaveMetrics Inc.) and MiniAnalysis 6 (Synaptosoft Inc.) or PClamp 10 (Molecular Devices Corporation). PSCs with amplitudes less than twice the noise level were excluded. All electrophysiological recordings were obtained at RT. Slices for these analyses were obtained from a total of ~100 animals, and all measurements reported are based on values obtained from at least three slices obtained from animals taken from distinct litters. More typically, data were obtained from 8-12 slices from 5-7 animals from distinct litters. All data recorded were used for analysis.

**Imaging**

Migration of Pax2-cells was observed in lobules IV and V of a total of 14 acute slices (300 µm) prepared from 14 animals from distinct litters. Slices were kept in modified, 35°C-warm ACSF containing (in mM): 132 NaCl, 3 KCl, 1.25 NaH2PO4, 2 MgCl2, 2 CaCl2, 20 NaHCO3, and 10 glucose. We blocked pre-synaptic release by adding either TeNT (100 µg/ml; 3 slices) or Co2+ (2 mM; 3 slices) to ACSF. One slice was used for sham incubation, and one for long-term observations (up to 4 h) to check for run-down of our preparation. To block post-synaptic receptors, we combined antagonists against ionotropic glutamate receptors of the AMPA/kainate- (NBQX, 20 µM) and NMDA subtypes (D-APV, 25 µM), metabotropic glutamate receptors (group I-III: LY341495, 5 µM; mGluR5: MPEP, 10 µM; mGluR1: 3-MATIDA, 40 µM), ionotropic GABAA receptors (Gabazine, 20 µM), metabotropic GABAB receptors (CGP55845, 4 µM), ionotropic purinergic receptors (P2X1-6: PPADS, 200 µM), metabotropic purinergic receptors (P2Y1-12: Suramin, 100 µM; P2Y1,2,4,6,,11,12: MRS2279, 0.5 µM), cytokine receptors including Trk A/B/C, p75 and others (NGF inhibitor: Ro 08-2750, 5 µM; pan-Trk inhibitor: GNF5837, 0.5 µM; JAK2, FLT3 and TrkA inhibitor: Lestaurtinib, 0.5 µM). For these pharmacological studies, we analysed cells from a total of 6 slices from 6 animals from distinct litters.

Time-lapse recordings (TLR) were done either in x,y,t (3 D) with a confocal LSM5-Pascal (Zeiss; Argon laser (Lasos), excitation at 488 nm), or in x,y,z,t (4 D) with an LSM TCS-SP5 (Leica) and two-photon excitation (pulsed IR laser, excitation at 980 nm; MaiTai BB, Newport/Spectra Physics). For 3 D TLR, images (217 × 217 µm2) were taken every 10 s for up to 4.5 h from an individual Pax2-cell, which was manually kept in the focal plane. In 4 D TLR, volumes of 456 × 456 × 30 µm3 were imaged every 42 s up to 4 h. If the z-drift was > 4 µm, z-settings were re-adjusted. Offline xyz-drift-correction was based on the stationary Golgi-cells. For quantitative analysis of Pax2-cell migration, centres of mass calculated from isosurfaces were tracked over time (Imaris 7.5.1, Bitplane). Only cells that were visible during at least 80% of the observation time were included. Speed was calculated from the distance the cells travelled between two successive observation points. Displacement represents the straight line distance between start- and end-point of a cell’s trajectory, and directionality is the ratio of displacement and length of the entire trajectory.

Angles between subsequent migratory steps were measured in the sagittal plane (i.e., the plane of slice sectioning) and in the coronal plane. For the calculation of probability density plots, we accounted for the fact that angular changes measured in the sagittal plane are continuous at + radians, and those in the coronal plane at + 0.5 radians. Data-based smoothing kernels were determined as described by Sheather and Jones (Jones et al., 1996; Sheather, S.J. and Jones,M.C., 1991).

**Electron microscopy**

Transgenic mice expressing EGFP under the control of Pax2 promoter were anesthetized and perfusion fixed transcardially with 0.01 M phosphate buffered saline (PBS; pH 7.4) containing 4% paraformaldehyde (PFA) and 0.05% glutaraldehyde at postnatal day 8. Cerebella were sectioned using a vibratome (50 µm thickness). Pre-embedding immune-peroxidase staining and electron microscopy were done as previously described (Boulland et al., 2003). Briefly, sections were treated with 1 M ethanolamine-HCl (pH 7.4) and 1% H2O2, followed by incubation overnight with anti-GFP antibodies (rabbit; 1:1000; Rockland Immunochemicals) in 10% newborn calf serum in 0.1 M Tris / HCl (pH 7.4) containing 0.3 M NaCl. The sections were then incubated with biotinylated anti-rabbit antibodies, streptavidin-peroxidase complexes, and finally with diaminobenzidine and 0.1% H2O2. Samples of stained tissue were dissected out, treated with 1% OsO4 in phosphate buffer, dehydrated in graded ethanols and propylene oxide and embedded in Durcupan ACM. Subsequently, ultrathin sections were cut and lightly contrasted with 2% uranyl acetate and 0.3% lead citrate. The samples were examined with a Tecnai CM10 electron microscope (FEI Company, Hillsboro, OR, USA).

Alternatively, the DAB-reaction product from immune labelling for GFP was intensified with a modified gold-substituted silver peroxidase technique (van den Pol and Gorcs, 1986). After washing DAB-reacted slices with 0.1 M Na-cacodylate buffer (CB; pH 7.6), they were fixed in 0.2% glutaraldehyde in CB and then osmicated in 0.1 M CB with 1% OsO4 and 1.5% potassium hexacyanoferrate for 15 min. After dehydration in a in a graded series of ethanols, they were flat-embedded in Epon. Semithin sections (1 μm) were cut on an LKB Ultratome, stained with Toluidine Blue and used for orientation. Ultrathin sections (50‑100 nm) were collected on Formvar-coated grids, counterstained with uranyl-acetate and lead-citrate, and examined with a Philips CM 100 transmission electron-microscope (FEI).

**Immunohistochemistry**

PFA-fixed slices (50 µm) were blocked with 2% normal goat serum in PBS for 2 h and incubated at 4°C with antibody G95 against synaptophysin (1:500) (Ikin et al., 1996) or against Calbindin D28K (Swant, Bellinzona, CH; 1:4000) overnight . Secondary Alexa-546 goat anti-rabbit or goat anti-mouse antibodies (1:500; Molecular Probes) were applied for 2 h. Confocal images were acquired using a Leica TCS SP2-system (excitation: 488 nm/543 nm; z-width 115 nm). To obtain a measure of the density of pre-synaptic elements in cerebellar layers, we measured the fraction of synaptophysin-positive pixels separately for each layer. To assess whether Pax2-cells might be contacted by synaptophysin-positive terminals, we first generated a mask based on the EGFP-signal to delineate Pax2-cells. Next, co-localization of EGFP and synaptophysin-immunoreactivity was quantified as described by Costes et al. (Costes et al., 2004). Computation was done with Imaris.

**Reagents**

NBQX, D-APV, LY 341495, (S)-3,5-DHPG, Lestaurtinib, GNF 5837, Ro 08-2750, PPADS, Suramin, CGP 55845, MRS2279, 3-MATIDA, MPEP, Gabazine and ionomycin were from Tocris; CNQX, bicuculline and muscimol from Ascent Scientific; DL-APV, KA, CTZ, GABA and picrotoxin from Sigma; TTX from Alomone Labs; and tetanus toxin from Calbiochem.

**Data analysis**

Summary data are given as means ± SEM unless otherwise stated. Experimental groups were tested for normality using Shapiro/Wilk and subsequently compared either using ANOVA followed by Tukey's HSD for Gaussian data; or Kruskal-Wallis followed by Dunn/Holland/Wolfe for non-Gaussian data. Speed, displacement and directionality were compared based on their medians to account for their non-normal distribution. Effect size was calculated as follows:

effect size (%) =

Percentile-based 95% confidence intervals for medians (Fig. S5D) and effect sizes (Fig. 6) were obtained by bootstrapping using 3000 repetitions. Angular distributions were compared applying Kuiper’s test. Test power was estimated post-hoc using GPower 3.1 if applicable. All statistical procedures were implemented in R (R Development Core Team, 2017).

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**Competing interests**

No competing interests declared.

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**FIGURE LEGENDS**

**Fig. 1: Morphology and migratory patterns of Pax2-cells.** (A) A Pax2-cell (p8) migrating through the IGL. Yellow curve: actual migratory path (length, 22.6 µm) reconstructed from 3D-images taken every ~42 s, and based on the cell's centre of mass (blue dots). Green arrow: displacement of the same cell (18.5 μm). White dots: centres of mass of three Pax2-cells that did not migrate during the observational period. See also Movies 1 and 2. (B) Migrating Pax2-cell. Single confocal plane, optical thickness 4.5 µm. (C) Pseudo-3D plots of a fast (red, left) and a slowly (blue, right) migrating Pax2-cell. Complete 3D stacks were recorded every ~42 s over 4 h. Each point gives the coordinates of the centre of mass over consecutive time points. (C’) Temporal variability of acute speed for the trajectories shown in C. (D) Summary view of speeds of 304 migrating Pax2-cells, recorded from an acute slice over 30 min in ~42 s intervals. Each color-coded rectangle represents the speed of individual cells at the indicated time points. Cells with comparable motility are arranged next to each other.

**Fig. 2: Morphological characteristics of Pax2-cells.** (A) Separate colour channels are shown for the Pax2-EGFP (left) and calbindin 28K signal (right) of Fig. 4A'. (B) Depicted are morphological characteristics used to identify migratory Pax2-cells en route to the molecular layer. These are maximum intensity projections of 60 optical sections through a volume of 375 \* 375 \* 21 µm3 of the nascent cerebellar cortex (p8; lobule 5; position equivalent to the boxed area in Fig. 4A). Exemplary cells identified as (resident) inhibitory interneurons of the granule cell layer are marked by yellow (vertical) arrowheads. Examples of cells in transit to and through the molecular layer are pointed out by red (horizontal) arrowheads. This initial identification based on morphology was corroborated by actual observation of the mobility, or immobility, of these cells as well as by electrophysiology (see main text). The position of the Purkinje cell layer is indicated by blue stars (based on nuclear counterstaining). Note that cells in transit to and through the molecular layer show strong EGFP expression. Strongly EGFP-positive cells can also be seen at the border between the external granule cell layer (EGL) and the nascent molecular layer (ML), where newly arrived Pax2-cells initially accumulate. In contrast, more mature inhibitory interneurons in the lower parts of the ML show only a very week (dim grey) residual EGFP signal. The EGL may be used as a reference for the EGFP-related background signal. The right hand panel shows nuclear counterstaining (propidium iodide) to document overall cell density and to facilitate identification of cerebellar cortical layers. The region shown corresponds to the left margin of the left hand panel.

**Fig. 3:** **Pax2-cells receive GABAergic and glutamatergic synaptic input.** (A) Whole-cell current pattern of a Pax2-cell (voltage steps from −70 mV to −160 – +20 mV, 10 mV increment, 50 ms duration, inset), indicating activation of voltage-gated K+ and Na+ channels (n = 176 cells analysed). See also Fig. S1. (B) Pax2-cells fire spikelets upon current injection. Cells pre-hyperpolarized to −70 mV were injected for 50 ms with up to 72 pA, in 8 pA increments (inset: current protocol). The histogram at the left gives the frequency of spikelet peak-voltages for a total of 25 cells analysed. (C) Inward currents induced by GABA (100 μM; holding potential –70 mV; top; n = 14 cells analysed plus n = 13 with application of the GABAA-receptor agonist muscimol) were blocked by bicuculline (100 μM, bottom; n = 4 cells each analysed with GABA and muscimol). (D) 300 μM KA induced an inward current at −70 mV (top; n = 14 cells analysed) that could be blocked with NBQX (10 μM, bottom; n = 8 cells analysed). See also Fig. S2. (E–G) Voltage-clamp recordings from a Pax2-cell in the middle ML held at –80 mV revealed rare sPSCs. Based on their current decay, these could be classified as fast or slow. An individual fast sPSC is shown in panel F (left; 11 pA, rise 0.3 ms, decay 0.6 ms). The right hand trace in panel F shows averaged fast sPSCs (17 pA, rise 0.7 ms, decay 1.5 ms, n = 4). An individual slow sPSC is shown in panel G ( left; 23 pA, rise 0.7 ms, decay 19.4 ms), and averaged slow sPSCs are depicted on the right hand side of panel G (23 pA, rise 0.9 ms, decay 12.1 ms, n = 20). (H,I) 10 µM bicuculline blocked slowly decaying, but not fast sPSCs. (i, left: individual sPSC, 26 pA, rise 0.6 ms, decay 0.7 ms; right: averaged sPSCs, 23 pA, rise 0.6 ms, decay 1.4 ms, n = 9). (J) 50 μM DL-APV and 10 μM NBQX inhibited the rapidly decaying sPSCs. (K) Histograms of decay times for fast (top; n = 490 from 43 cells) and slow sPSCs (bottom; n = 1915 from 31 cells). (L–N) Recordings from a cell that had been observed to migrate immediately prior to analysis (n = 32 cortical and n = 3 Pax2-cells in WM analysed for postsynaptic currents after proven migration; n = 3 cells analysed in cortex plus n = 3 cells in WM with TTX and ionomycin). (L) Voltage-clamp (−80 mV) recordings in ACSF (top trace). 1 µM TTX isolated mPSCs (middle trace), the frequency of which was strongly increased by 3 μM ionomycin (lower trace). (M) Averaged fast (left, 13 pA, rise 0.5 ms, decay 2.6 ms, n = 72) and slow (right, 6 pA, rise 1.6 ms, decay 17 ms, n = 22) mPSCs recorded in ionomycin. (N) Cumulative probability density of sPSC-rates in ACSF (black), in TTX (grey), and with TTX plus ionomycin (red; from traces shown in L). TTX reduced, and ionomycin increased sPSC rates. Scale bars in E apply also to H,J; those in G also to F,I,M). See also Fig. S3.

**Fig. 4: Distribution and density of Pax2-cells and pre-synaptic elements in the nascent cerebellar cortex.** (A,A') Parasagittal section through the cerebellar vermis of a p8 animal shows the distribution of EGFP-positive Pax2-cells (green) throughout all layers except the external granule cell layer (EGL). Purkinje cells are stained for calbindin 28K (magenta) and help to delineate the nascent molecular layer (ML) and the Purkinje cell layer (PCL). Deep nuclei (DN) are also magenta due to the presence of Purkinje cell axon terminals, as is the white matter (WM) traversed by Purkinje cell axons. Yellow box symbolizes how panels A’ and B relate to this overview. Roman numerals indicate cerebellar lobules. (A‘) Higher magnification focusing on the cerebellar cortex. IGL, internal granule cell layer. For separate colour channels of this panel, see Fig. 2A. (B) Staining for synaptic vesicles (synaptophysin, magenta). Boxes indicate areas shown at higher magnification in B‘. (B‘) Synaptophysin-positive puncta (examples indicated by arrows) can be found close to Pax2-cells (green) throughout the nascent cerebellar cortex (lobule IX; p8). (C) Layer specific quantification of synaptophysin positive puncta. Staining density in the nascent ML is consistently higher than in all other layers and the WM (tests: Shapiro-Wilk p > 0.05, ANOVA p = 5.5 10-5 followed by Tukey p < 0.001. 4 slices were analysed). (D) Quantification of synaptophysin positive puncta over a mask defined by Pax2-cells shows how potential synaptic contacts close to Pax2-cells increase from the WM to the ML. Numbers of cells analysed are given in parenthesis (tests: Shapiro-Wilk p < 0.003, Kruskal-Wallis p ~ 10-30, followed by Dunn-Holland-Wolfe test, p < 0.001, n = 4 slices were analysed).

**Fig. 5: Ultrastructural details of Pax2-positive cerebellar cells.** (A) Cells immuno-positive for EGFP expressed from the Pax2-locus (termed Pax2-cells, outlined using the blue overlay) surrounded by neuropil characteristic for the molecular layer. A Purkinje cell perikaryon (pink) is nearby, confirming the position of the Pax2-cells in the lower molecular layer. Pax2-cells display several protrusions, and they are contacted by presynaptic elements. Such synapses are shown at higher magnification in B and C. (B) Higher power view of the upper boxed area from panel (A) shows two presynaptic elements (T1, T2) contacting the Pax2-cell (P1). (C) Higher power view of the lower boxed area from (A) shows one broad synapse (T3) contacting the Pax2-cell (P2). Note post-synaptic densities on Pax2-cells (bordered by arrow heads) and clusters of synaptic vesicles in nerve terminals apposing them (asterisks). Arrows indicate peroxidase deposits. Scale bars: 10 µm in A, 0.5 µm in B,C. See also Fig. S4.

**Fig. 6: Synaptic innervation directs the migration of Pax2-cells.** Block of pre-synaptic release with tetanus toxin (TeNT) or Co2+ consistently and reproducibly reduced cell displacement, directionality and speed. Shown are medians of effect sizes and their bootstrap-based 95% confidence intervals. For each condition, three slices derived from three animals were analysed, comprising a total of 882 (TeNT) and 1130 (Co2+) cells, respectively. The sham slice documented was incubated for 2 hours (i.e., as TeNT-incubated slices, but omitting TeNT) in order to identify potential "run-down" effects due to protracted incubation. No such effects could be detected (total of 262 cells). In contrast, bath application of a cocktail of receptor antagonists blocking ionotropic and metabotropic receptors for glutamate and GABA, purines (P2) and also neurotrophin receptors affected migration only moderately and showed high intra- and inter-experimental variability. Data were obtained from six slices of six animals encompassing a total of 2069 cells. Note: Effects are significant if their confidence interval does not include zero (dashed line). The colour code indicates whether treatment significantly reduced (green dots), increased (black dots), or did not change (magenta dots) the parameter analysed. See Fig. S5 for additional data and methodological details.

**Fig. 7: Synaptic innervation enhances directional persistence of migrating Pax2-cells.** Typical probability density maps of between-step turning angles (Δφ) of Pax2-cells migrating in acute slices of p8 cerebella. Δφ values were determined as projections in the sagittal (Δφx,y) and coronal (Δφx,z) planes (see Fig. S6 for details). The probability density of individual Δφ values is displayed along the z-axis (inset: colour code). Under control conditions, many Δφ values were close to 0 resulting in a central peak. (A,C) Incubation with tetanus toxin (A, 100 μg/ml) or wash in of Co2+ (C, 2 mM) abolished the central peak (A‘,C’). This indicates decreased directional persistence. (A‘‘,C’’) Δφxyz angles, determined in 3D before (black traces) and after (magenta traces) incubation with TeNT (A’’) or Co2+ (C’’). Thus, blockade of synaptic release results in a clear-cut shift of turning angles towards higher values (Kuiper’s test, p < 0.001). (B) Sham incubation without TeNT had no substantive effect on the central peak (B‘) or on the distribution of Δφxyz angles (B‘‘) (Kuiper’s test, p = 0.221). Between 7510 and 12740 Δφ values were analysed per condition.

**Fig. 8: schematic view how transmitters might impinge on the mobility of neuronal precursors.** Grey arrows symbolize transmitter action – effect size is symbolized by arrow size. Under physiologic conditions, and also with TeNT, isotropic, paracrine input keeps the cell in a "receptive" state, i.e. its internal machinery is plastic and responsive to directive signals (which may come from synaptic input). In contrast, in the absence of all input, this plasticity is lost, and the cell initially persists in the state it had immediately before receptor blockade. Eventually, this internal state decays, and cell movement also becomes erratic.