Neuronal survival during mammalian development crucially depends on target-derived neurotrophic factors. Target loss removes this trophic support and leads in most cases to the transsynaptic retrograde degeneration of the respective afferents. Primary vestibular afferents (PVA) originating from bipolar neurons in the vestibular ganglion (VG) are the first mossy fibers that enter the cerebellum, but little is known about the survival requirements of VG neurons. In the present study the influence of the differential granule cell (GC) target loss on the survival of VG neurons was studied quantitatively using unbiased stereological methods in the cerebellar mutants Purkinje cell degeneration (pcd/pcd), Lurcher (Lc/+), and Weaver (we/we). Neither the secondary GC loss in the Purkinje cell deficient mutants pcd/pcd and Lc/+, nor the primary loss of GCs in we/we produced any significant reduction in the total number of bipolar neurons in the VG compared to controls. So, PVA neurons are highly resistant to cerebellar target deprivation and survive in the absence of cerebellar granule and Purkinje cells, regardless of whether the target loss occurs before (in we/we), during (in Lc/) or after (in pcd/pcd) the mossy fiber–granule cell synaptogenesis. NeuroReport 9: 4119–4122 © 1998 Lippincott Williams & Wilkins.

Key words: Lurcher; Primary vestibular afferents; Purkinje cell degeneration; Scarpa’s ganglion; Stereology; Target deprivation; Transsynaptic degeneration; Weaver

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Introduction

Trophic reciprocal interactions between target neurons and their afferents are thought to regulate neuronal survival and to determine the shape and size of axonal and dendritic processes during development.1,2 However, it is unclear whether afferents that innervate more than one target neuronal population need to be trophically supplied by all these populations to survive or whether a hierarchy among targets exists. Thus, the loss of the main target possibly leads to cell death, whereas removal of minor projection sites may cause only somatic and/or terminal modifications or even not affect the morphology of afferents at all. Moreover, in mature neurons the importance of a continued target-derived trophic support has been questioned, as only several neurons maintain a dependence on target integrity for their continued survival while others apparently do not.3

The brainstem vestibular nuclei and the cerebellum constitute the two main central targets of primary vestibular afferents (PVA), and it has been shown recently that in mutant mice lacking brain-derived neurotrophic factor (BDNF), vestibular symptoms evolve and excessive degeneration of the vestibular ganglion (VG) neurons occurs despite previous normal ganglion formation, extension of nerve processes and synaptogenesis.4 In the present study, the impact of the loss of one of these targets, the cerebellar granule cells (GC), on the survival of primary vestibular afferent neurons in the VG has been investigated using cerebellar mutant mice with different developmental time courses of granule cell degeneration: In weaver mutants (we/wv) GCs intrinsically die before synaptogenesis with mossy fibers.6 In Purkinje cell degeneration (pcd/pcd) mutants the transsynaptic GC loss is preceded by normal synapse formation between GCs and mossy fibers and in Lurcher (Lc/) mutants the loss of GCs parallels the establishment of synaptic contacts.10

Unbiased stereological disector methods11 and morphometry have been used to estimate the total number and size of VG neurons in these mutants.

Materials and Methods

pcd/pcd, Lc/+ and we/wv mutant mice were derived from breeder pairs originating from Jackson Laboratories (Bar Harbor, ME, USA) and kept under a...
normal dark/light rhythm with food and water ad lib. Three mutants of each strain, aged 6–9 months, were perfusion-fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M PO4 buffer at pH 7.4 and 4°C for 30 min preceded by 1.75 g/kg chloral hydrate anaesthesia and perfusion with normal saline at 37°C. The entire bony labyrinths were dissected immediately thereafter and decalcified for 5 days in 5% EDTA at pH 7.4 and 4°C. Subsequently, the labyrinths were plastic-embedded in araldite and the entire vestibular ganglia sectioned in the mediolateral axis on a LKB-ultratome at 1 µm. Sections were stained with Richard’s solution.

For stereological analysis the physical disector was used. The appropriate disector height (the distance between reference and look-up section) was set at 5 µm, since the smallest minimum diameter of more than 150 (~50 from each mutant strain) completely reconstructed neurons was 10.2 µm. Systematic random sampling proceeded as follows. The initial disector was randomly chosen within the first 10 sections containing the ganglion, continuing with the next disector every 15 µm to the distal end of the ganglion. This resulted in a mean of 19 dissectors per ganglion. The VG neurons included in the look-up but not in the reference section were counted over the complete cross-sectional area of the ganglion. In order to achieve the volume sampling fraction, the area of the estimated look-up sections was computed with a digitizing tablet, added and multiplied by the disector height (5 µm). Finally, the total number was calculated from the relationship between the neurons of the volume sampling fraction and the complete volume of the respective ganglion. The total volume of the ganglia was determined according to Cavalieri’s principle (for review see Ref. 11) by adding the cross-sectional areas of the ganglion in all sections and multiplying by the section thickness.

The data from two different wild-type strains (C57BL/6j and B6CBA), published earlier, served as controls for the present estimates from cerebellar mutants.

Results

The total number of VG neurons (Table 1) was 3526 ± 380 in pcd/pcd, 3430 ± 88 in Lc/+ and 3387 ± 331 in wv/wv. There were no significant differences present compared with wild-types with a mean total number of 3433 ± 232 nor when comparing the different mutant strains one with another.

The mean total VG volumes (Table 1) were 0.018 mm³ in pcd/pcd, 0.017 mm³ in Lc/+ and 0.016 mm³ in wv/wv, which is not significantly different from wild-types (0.018 mm³). In Figure 1 the maximum

| Strain  | No. | Total number | Coefficient of error | Total volume (mm³) |
|--------|-----|--------------|----------------------|--------------------|
| pcd/pcd| I   | 3859         | 0.24                 | 0.018              |
| pcd/pcd| II  | 3822         | 0.23                 | 0.019              |
| pcd/pcd| III | 3997         | 0.17                 | 0.017              |
| mean   |     | 3526 (= 380) | 0.21                 | 0.018              |
| Lc/+   | I   | 3925         | 0.14                 | 0.018              |
| Lc/+   | II  | 3350         | 0.21                 | 0.016              |
| Lc/+   | III | 3417         | 0.11                 | 0.017              |
| mean   |     | 3430 (= 88)  | 0.15                 | 0.017              |
| wv/wv  | I   | 3596         | 0.07                 | 0.018              |
| wv/wv  | II  | 3055         | 0.11                 | 0.015              |
| wv/wv  | III | 3562         | 0.08                 | 0.016              |
| mean   |     | 3387 (= 331) | 0.08                 | 0.016              |

1Coefficient of variance (CV) = 0.10 in pcd/pcd; 0.02 in Lc/+, and 0.09 in wv/wv. Standard deviations are given in brackets.

rostralcaudal extent of the VG of a wild-type mouse and the cerebellar mutants is shown. No significant differences were observed either in the rostralcaudal or in the mediolateral and dorsoventral planes between wild-types and mutants or among the mutants. The pooled mean maximum extensions were: rostrocaudally 705 ± 101 µm (wild-types 696 µm), mediolaterally 417 ± 84 µm (wild-types 418 µm), and dorsoventrally 362 ± 40 µm (wild-types 377 µm).

The minimum diameter of the smallest neurons was 10.2 µm and the maximum diameter of the largest was 27.2 µm (pcd/pcd = 27.6; Lc/+ = 27.1; wv/wv = 26.9) which differed significantly from wild-types (11.5 µm and 31.8 µm, respectively). No signs of degeneration were observed in any of the ganglia.

Morphometry of the somatic cross-sectional areas of VG neurons in the superior (SD) and inferior divisions (ID) of the ganglion (Fig. 2) revealed somatic atrophy of VG neurons in the mutants when compared to wild-types. Cellular atrophy is highly significant in all mutant strains in the SD, whereas in the ID the cross-sectional areas of VG neurons are reduced only in wv/wv.

Discussion

Labyrinthectomy with or without direct injury to the peripheral axonal portion of the VG neuron (the dendrite) is not sufficient to produce degeneration of VG neurons. In contrast, neurectomy central to the ganglion causes death of bipolar neurons and the lack of BDNF without direct lesion to PVA produces massive degeneration in the VG. Thus, trophic interaction of PVA with central rather than peripheral targets seems to be of preponderant importance for the survival and maintenance of VG neurons. However, more than one central target of direct PVA innervation exists: fibers projecting to the brainstem vestibular nuclei bifurcate and send collaterals to the
vestibulocerebellum, where they end as mossy fibers on granule cells and to a minor extent also on Golgi cells. PVA terminations are also described in parts of the reticular formation and the cerebellar nuclei (for review see Refs 15 and 19). The question is whether all of these targets are essential for the survival of VG neurons. The present results in \( wv/wv \) mutants, with their complete GC loss in the vestibulocerebellum, where they end as mossy fibers on granule cells and to a minor extent also on Golgi cells. PVA terminations are also described in parts of the reticular formation and the cerebellar nuclei (for review see Refs 15 and 19). The question is whether all of these targets are essential for the survival of VG neurons. The present results in \( wv/wv \) mutants, with their complete GC loss in the vestibulocerebellum, show that the GC target is not vital for VG neuron survival. However, in agranular cerebellum atypical mossy fiber contacts on Purkinje cell dendrites have been described.\(^{16}\) In these cases, the loss of trophic support from GCs has possibly been compensated by interactions with Purkinje cells. This can be excluded in \( pcd/pcd \) and \( Lc/+ \) mutants since Purkinje cells are completely missing, though VG neurons survive. Thus, not only the presence of GCs but also of Purkinje cells are inessential for the maintenance of VG neurons. In both Purkinje cell-deficient mutants GC loss is very severe: in \( pcd/pcd \) > 80% of GCs are lost between 3 and 12 months of age\(^ {17} \) and in adult \( Lc/+ \) mutants GC degeneration is 90%.\(^ {18} \) Thus, homotypical reinnervation of the few remaining GCs by the vestibulocerebellar afferents could theoretically suffice to regain their support, yet the degree of GC reduction casts some doubt on
whether all PVA without exception are successful in recuperating GC assistance in competition with all other mossy fibers. In fact, not a single VG neuron dies, and regressive phenomena rather than reinervative attempts of PVA have been observed in pcd/pcd and Lc/+ mutants. This is in remarkable contrast to the behaviour of the second main afferent population to the cerebellum, the inferior olivary climbing fibers (IOC): there is a massive degeneration of IOC in pcd/pcd and Lc/+ due to the deprivation of Purkinje cells. IOCs die despite the continued presence of their other regularly innervated synaptic counterparts, such as cerebellar interneurons and target cells in the deep cerebellar nuclei (for review see Ref. 22). Thus, the loss of the cerebellar target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target domain affects the survival of cerebellar afferents differentially: 50% of climbing fibers and their target.

VG neurons are born at embryonic day (E) 11–13 and PVA enter the cerebellum at E15. GCs migrate until postnatal day (P) 15 and mossy fibers contact only postmigratory GCs. In wv/wv GCs die pre-migratory and mossy fiber–granule cell synapses are never formed. In Lc/+, GC degeneration proceeds during the phase of GC migration and thereafter, and mossy fibers appear to make normal synapses on surviving GCs. In pcd/pcd GC loss occurs after normal mossy fiber–GC synapse formation. However, in all three mutants the VG neuronal number does not differ. In consequence, neither granule cell absence nor Purkinje cell loss have any significant effect on the survival of VG neurons. It seems rather that once generated and grown into the cerebellum, VG neurons and their vestibulocerebellar afferents do not need any further support from the cerebellum to endure. The sole morphological alteration in the VG in response to the loss of the cerebellar target domain is a reduction in cell size, which may reflect the concomitant decrease in the metabolic activity of these neurons. Combined with the results from BDNF-lacking mutants, these findings suggest that trophic support is provided most probably by vestibular nuclei neurons in the brainstem and that this support is sufficient to guarantee the long-term survival of VG neurons after direct GC–target loss and even after the additional loss of cerebellar Purkinje cells. The results show in addition that retrograde loss of VG neurons does not contribute to the vestibular symptoms observed in cerebellar ataxia.

Conclusion

VG neurons survive under complete and partial deprivation of granule and Purkinje cells and neither the formation nor the continued preservation of mossy fiber–granule cell synapses are essential for their maintenance.

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