Morphology and Physiology of the Cerebellar Vestibulolateral Lobe
Pathways Linked to Oculomotor Function in the Goldfish

Hans Straka,1 James C. Beck,2 Angel M. Pastor,3 and Robert Baker2
1Laboratoire de Neurobiologie des Réseaux Sensorimoteurs, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7060, Université Paris 5, Paris, France; 2Department of Physiology and Neuroscience, New York University School of Medicine, New York, New York; and 3Departamento de Fisiologia y Zoología, Facultad de Biología, Seville, Spain

Submitted 30 March 2006; accepted in final form 8 June 2006

Straka, Hans, James C. Beck, Angel M. Pastor, and Robert Baker. Morphology and physiology of the cerebellar vestibulolateral lobe pathways linked to oculomotor function in the goldfish. J Neurophysiol 96: 1963–1980, 2006. First published June 14, 2006; doi:10.1152/jn.00334.2006. Intracellular recording and single-cell labeling were combined to investigate the oculomotor circuitry of the goldfish cerebellar vestibulolateral lobe, consisting of the eminentia granularis (Egr) and caudal lobe. Purkinje cells expressing highly conserved vertebrate electrophysiological and morphological properties provide the direct output from the caudal lobe to the vestibular nuclei. Biocytin labeling of the Egr distinguished numerous hindbrain precerebellar sources that could be divided into either putative mechanosensitive or vestibulosensitive nuclei based on cellular location and axon trajectories. Precerebellar neurons in a hindbrain nucleus, called Area II, were electrophysiologically characterized after antidromic activation from the Egr (>$50\%$ bilateral) and their morphology analyzed after intracellular biocytin labeling ($n=28$). Bipolar spindle-shaped somas ranged widely in size with comparably scaled dendritic arbors exhibiting largely closed field configuration. Area II neurons ($85\%$) projected to the ipsilateral Egr with most ($93\%$) sending a collateral through the cerebellar commissure to the contralateral Egr; however, $15\%$ projected to the contralateral Egr by crossing in the ventral hindbrain. Axon terminals in the vestibular nuclei were the only collaterals within the hindbrain. Every Area II neuron received a disynaptic EPSP after contralateral horizontal canal nerve stimulation and a disynaptic IPSP, preceded by a small EPSP ($>50\%$), after ipsilateral activation. Vestibular synaptic potentials were of varying shape/amplitude, unrelated to neuron location in the nucleus, and thus likely a correlate of somadendritic size. The exceptional separation of eye position and eye velocity signals into two separate hindbrain nuclei represents an ideal model for understanding the precerebellar projection to the vestibulocerebellum.

INTRODUCTION

The cerebellum of teleosts has been divided into three subdivisions—corpus cerebelli, valvula cerebelli, and vestibulolateral lobe (VL)—that can be simultaneously seen in a parasagittal section to exhibit continuous granule, molecular, and Purkinje cell layers (Figs. 1, A and B and 3A). The vestibulolateral lobe has long been considered homologous to the cerebellar auricles of amphibians and reptiles (Larsell 1967), which themselves have been envisioned to be a forerunner of the mammalian vestibulocerebellum (Cohen and Takemori 1973; Lisberger and Fuchs 1978; Waespe et al. 1983). Acute experiments in goldfish that individually inactivated the three cerebellar subdivisions during vestibuloculard reflex (VOR) plasticity indirectly demonstrated VL to be in fact the cerebellar area related to eye and head movement signal processing (Michnovicz and Bennett 1987; Pastor et al. 1994a; Weiser et al. 1988). The role of VL was directly corroborated by recording both ipsilateral ($E_I$) and contralateral ($E_{II}$) horizontal eye velocity–related activity that, unlike comparable experiments in primates (Hirata and Highstein 2001; Lisberger et al. 1994; Miles et al. 1980), did not appear to change discharge sensitivity during VOR learning paradigms (Pastor et al. 1997).

The above findings strongly suggested that the dominant site for oculomotor plasticity in the goldfish was located within the brain stem, not the cerebellum. By contrast, the majority of prevailing views derived from mammalian species have centered around either intrinsic changes within the cerebellum (Raymond and Lisberger 1998) through the mechanisms of long-term depression and potentiation (Boyd et al. 2004; Ito 1989) or, in more recent years, multiple plasticity mechanisms/sites that exhibit the capacity to store memory over different timescales and contexts (Boyd et al. 2004). Despite the predominant focus on the cerebellum as the site for oculomotor plasticity, it has always been thought that resolving the site-specific cerebellar-dependent learning conundrum would depend on complementary knowledge of precerebellar neuronal pathways and signals (Miles and Lisberger 1981). Hence an attractive experimental model that could offer insight into these long-standing differences of opinion (Blazquez et al. 2004; Boyd et al. 2004; Highstein et al. 2005; Ito 2005; Raymond and Lisberger 1998) provided the rationale for delineating the afferent and efferent organization of the goldfish vestibulolateral lobe.

In most vertebrates, each cerebellar folia consists of a superficial molecular layer, an intermediate Purkinje cell layer, and a deep granule cell layer (Larsell 1967). However, the phylogenetic consistency of this cerebellar unit was first questioned in teleosts because of the apparent absence of distinct and localized deep cerebellar nuclei. A cell type identified near the junction of the molecular and granule cell layer that was initially termed “Assoziation-Zelle” (Franz 1911), and later called “eurydendroid cell” based on the shape of the dendritic tree, was envisioned to receive Purkinje cell axon terminations (Nieuwenhuys and Nicholson 1969; Pouwels 1978a), leading to the suggestion that “Purkinje-like” cells provided the direct cerebellar output (Finger 1978b; Pouwels 1978b). Accord-
ingly, the first objective of this study was to document the presence of Purkinje cells in the goldfish vestibulolateral lobe by recording their signature mossy and climbing fiber responses and visualizing a typical somadendritic morphology including axonal projections to the octaval nuclei (Highstein et al. 1992; McCormick and Braford 1994).

The major objective of this study was to identify the brain stem sources providing afferent input to cerebellar vestibulolateral lobe granule cells, in particular neuronal subgroups related to vestibular and oculomotor function. The VL in teleosts is a transitional structure between the corpus cerebelli and the dorsal medulla (Fig. 1, A and B) especially in species with electrorception (see Discussion) (Sas and Maler 1987). In the major work dedicated to the anatomical organization of the VL in nonelectroceptive cyprinids (Bass 1982), two sub-divisions called the eminentia granularis (Egr) and the lobus caudalis (LC) were observed in all species. Here, all afferent neurons to the Egr were mapped by a combination of retrograde tracing and single-cell–labeling studies designed to identify precerebellar brain stem afferents that collectively are the source of oculomotor (i.e., in addition to vestibular) signals in the LC Purkinje cells (Pastor et al. 1997). This effort uncovered a large precerebellar nucleus in the goldfish hindbrain (Figs. 1, B and F–I and 2, A and C) whose location coincided with the same area studied earlier (Pastor et al. 1994b). The activity of neurons in this brain stem area was shown to be correlated with eye velocity (Pastor et al. 1994b) and located just rostral to another group of neurons specific for signaling eye position (Fig. 1B). From a physiological perspective, both subgroups formed more or less compact nuclei with seemingly homogeneous physiological properties that, for the lack of an explicit compelling nomenclature, were initially termed Area I and Area II (Pastor et al. 1994b).

Subsequent morphology and physiology indicated that all neurons in Area I were of the classically defined burst/tonic type and well correlated with horizontal eye position (Escudero et al. 1996; Lopez-Barneo et al. 1982; McFarland and Fuchs 1992). Their axons projected to either the ipsilateral abducens nuclei or contralateral Area I/abducens nuclei in a presumed reciprocal excitatory and inhibitory pattern (Aksay et al. 2000, 2001). By contrast, preliminary anatomy showed that some Area II neurons sent axons to the cerebellum signaling contralateral horizontal head and ipsilateral eye velocity (HgEi) (Pastor et al. 1994b), but the explicit hindbrain and cerebellar connectivity, especially individual cellular morphology and electrophysiology, remained unclear.

The Area II neurons described here represent an extraordinary major brain stem source of mossy fiber signals to Purkinje cells in the cerebellar VL with projections not only to the ipsilateral, but also to the contralateral (Egr) granule cell layer including substantial collaterals to the vestibular-related divisions of the octaval nuclei (Pastor et al. 1997). Based on a comprehensive, single-cell description of their morphology and electrophysiology the present study establishes a close evolutionary and developmental relationship between Area II and vestibular neurons that in turn provides a structural counterpart for quantitative analysis of their neurophysiological signaling during oculomotor performance and plasticity in the alert goldfish (Bassett et al. 2005; Beck et al. 2001, 2006).

Preliminary results were previously published in abstract form (Straka and Baker 1998).

**METHODS**

**General and surgical procedures**

Experiments were performed on 46 adult goldfish (Carassius auratus) with a size of 15–20 cm, obtained from an institution-approved supplier (Hunting Creek Fisheries, Thurmont, MD) and maintained at 18°C in aquarium exposed to a 12:12 h light/dark cycle. Experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NRC 1996). The New York University School of Medicine Institutional Animal Care and Use Committee approved specific protocols.

Two days before an acute experiment, goldfish were deeply anesthetized in tricaine methanesulfonate (MS222; 1:2,000 wt/vol; Sigma, St. Louis, MO). Self-tapping screws were placed in the skull and stabilizing bolts were anchored with dental acrylic. To obtain orthodromic identification of Area II neurons, Teflon-coated silver wires were implanted on both horizontal semicircular canal nerves by opening the bone flap above the operculum and removing the muscle to reveal the lateral part of the labyrinth, which was carefully opened to expose the horizontal canal ampulla and nerve. A twisted pair of insulated silver wires (100 μm diameter) were placed with a micro-manipulator on the nerve close to the ampulla and fixed to the skull with dental cement. The opercular cavity was closed with a piece of tissue fixed with an adhesive (3M, Vetbond). To directly visualize the cerebellum and hindbrain for recording neuronal activity a 5 × 5-mm window was made in the bare skull roughly centered on the occipital crest between, but not encroaching on, the semicircular canals. The excised bone flap was refitted and then sealed with the tissue adhesive.

All experiments that included electrical stimulation were carried out in anesthetized goldfish that received pentobarbital sodium (20–30 mg/kg) and gallamine triethiodide (Flaxedil; 1–2 mg/kg). Goldfish were wrapped in protective cotton gauze, placed in a white acrylic aquarium (27 cm diameter) filled to a level below the cranium, and gently restrained by two C-shaped clamps that were attached to the wall of the tank. Stabilization was achieved by connecting the head to the aquarium by a small aluminum plate, affixed to the bolts implanted 2 days before the experiment. Recirculating aquarium water was filtered, aerated, and maintained at 18°C with a thermoelectric device. At the beginning of each experiment, the bone flap above the
FIG. 2. High-magnification coronal views of Area II, vestibulolateral lobe granule cell layer, and pontine and vestibular nuclei. A: coronal view at the level of Fig. 1H showing the highest density of ipsilateral Area II neurons including scattered, dorsally located cells (green arrows) that may also be ventral reticular neurons. B: coronal view of the ipsilateral PN with both dorsal and ventral axons indicated by black arrows. C: caudal contralateral Area II nucleus (cAII) at the level of Fig. 1I showing axons in the vHCom and a close, nearly continuous, relationship with the inferior olivary nucleus (IO). A dorsal reticular neuron possibly located in the region of Area I is indicated by a black arrow. D: high-magnification view of the dorsomedial and narrower ventral part of the descending octaval (DO) nucleus at the level of Fig. 1F showing labeled neurons (blue arrows). E: bilateral termination pattern in the vestibulolateral granule cell layer (Egr, white dashed ovals) after biocytin application to the ipsilateral Area II nucleus (not illustrated). F: ipsilateral Egr label after biocytin label of the ipsilateral horizontal canal nerve. Red arrows show the IO and Purkinje axonal pathways. Abbreviations as in Fig. 1.
recording area was loosened and removed from the head. Antidromic activation of Area II neurons was achieved by a twisted pair of Teflon-coated silver wires (75 μm in diameter) either inserted about 2.0 mm into the VL or, more frequently, placed adjacent to the VL with a micromanipulator. Purkinje cells were antidromically activated by electrical stimulation through electrodes placed close to the vestibular nuclei. To efficiently access the more rostral Area II with frequent electrode insertions, the caudal part of the facial lobe was removed by suction.

**Recording**

Electrodes for intracellular recordings were fabricated with a Narishige vertical puller and filled with either 2 M potassium acetate (30–50 MΩ) or 5% neurobiotin (Vector) in 0.5 M potassium acetate (35–60 MΩ). At the beginning of each recording session, antidromic and horizontal canal nerve–evoked synaptic field potentials were recorded and used as searching stimuli to recognize the target site. To fill intracellular recorded neurons with neurobiotin, positive current (4–10 nA) with a duty cycle of 50% at 2–4 Hz was passed through the recording electrode for 2–10 min. More detailed procedures are presented in Aksay et al. (2000) and Graf et al. (1997).

**Retrograde tracing of afferent inputs to the vestibulocerebellum**

The location and total extent of the hindbrain neuronal populations that project to the vestibulocerebellum were anatomically identified by placement of biocytin crystals (Molecular Probes) into the Egr of the vestibulolateral lobe (Fig. 1C). For these experiments (n = 9) goldfish were deeply anesthetized in MS222 and prepared for the tracer application using a lateral approach through the labyrinth as described for horizontal canal nerve stimulation. After opening the labyrinth and revealing the endorgans, the medial wall of the labyrinth separating the latter from the brain was opened above the vestibulolateral lobe with forceps. The lateral aspect of the Egr was identified (see Fig. 1A), a small cut was made with fine tungsten wires, and biocytin crystals were placed into the incision. After removal of surplus tracer, the opercular cavity was closed with a piece of tissue fixed with the adhesive (Vetbond).

**Histological procedure**

Animals were allowed to survive for 14–24 h after intracellular neurobiotin staining and for 48 h after biocytin application into the vestibulocerebellum in a tank with aerated fresh water of 18°C. Thereafter, goldfish were again deeply anesthetized in MS222 and transcardially perfused with 10–15 ml freshwater teleost Ringer followed by 100 ml of an aldehyde fixative (4% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4). After removal, brains were postfixed for 24–36 h and subsequently stored in a 0.1 M phosphate-buffered (PB) 30% sucrose solution. Brains were cut coronally in 75–μm sections from the midbrain to the spinal cord. Sections were incubated in a solution containing 0.3% hydrogen peroxide in 70% methanol and then washed again in 0.1 M phosphate buffer (pH 8.0). After washing in 0.1 M PBs and in 0.05 M Tris buffer (pH 7.4) sections were incubated for 8 min in a solution containing 0.04% 3’3-diaminobenzidine, 0.4% ammonium nickel sulfate, and 0.02% hydrogen peroxide in 0.05 M Tris buffer (pH 8.0). Some of the initial single-cell label experiments were viewed without intensification by ammonium nickel sulfate (e.g., Fig. 3). Subsequently, sections were washed in cold rinse Tris buffer (pH 8.0), mounted on gelatin-coated slides, dried overnight, counterstained with cresyl violet, and coverslipped. After histological processing of 15 Purkinje cells and 38 Area II neurons labeled by single-cell injections, visual reconstruction of consecutive sections showed that the axonal projections, including the location and morphology of the somadendritic tree, were sufficiently complete in eight and 25 neurons, respectively. Two Purkinje cells (Fig. 3) and four Area II neurons (Figs. 4–8) were selected as particularly representative of the physiological characteristics, and seven Area II neurons with a well-identified axonal projection, clearly visible cell body, and dendritic tree were reconstructed with a ×40 oil-immersion objective (Figs. 5–9). Sections were either photographed and then scanned or digitized directly with an AxioCam camera before processing with Adobe Systems Graphics.

**RESULTS**

**Afferent organization of the vestibulolateral lobe**

The key labeling approach that distinguishes the present findings from all previous studies (Finger 1978a; Ikenaga et al. 2002) was lateral visualization of the Egr of the vestibulolateral lobe allowing a selective, direct application of biocytin crystals (n = 8; Fig. 1, A–C). The results are illustrated in five comparably sized hindbrain coronal sections (Fig. 1, E–I) and a full hindbrain/cerebellar coronal section showing the Egr biocytin label (Fig. 1C). Three distinct, precerebellar rostral hindbrain nuclei (pontine, PN; subminentialis, sE; anterior octaval, AO) were delineated and their locations are depicted in Fig. 1D.

A major observation after biocytin Egr application on one side was that retrograde labeling of hindbrain precerebellar nuclei occurred on both sides (Fig. 1, F–I). Axons of the contralaterally located neurons from either mechanosensitiv (lateral line) or vestibulosensitiv (canals and otoliths) second-order sensory nuclei neurons distinguished three distinct commissures (cerebellar, CbCom; dorsal hindbrain, dHCom; and ventral hindbrain, vHCom) as schematically illustrated in Fig. 1D (see DISCUSSION). The cerebellar commissure (also known as Larsell’s transverse plate; see DISCUSSION) lay directly ventral to the valvula cerebelli granule cell layer at the rostral end of the vestibulolateral lobe (Fig. 1E) where the granule cell layers of the corpus cerebelli and vestibulolateral lobe appear continuous (level E in Figs. 1A and 3A). The CbCom appears to be the major conduit for the hindbrain vestibular and Area II subgroups as illustrated most clearly by the reconstructions shown in Figs. 5–9.

Commisural fibers also cross below the ventricular surface of the dorsal hindbrain (Fig. 1, D–F, dHCom) as well as just above the pial surface of the ventral hindbrain (Figs. 1, D, H, I and 2, A and C, vHCom). The dHCom connects neurons between the medial octaval nucleus (MO) and the Egr (i.e., mechanosensitiv), whereas the vHCom is used exclusively by axons of the pontine (PN; Fig. 2B), descending octaval (DO; Fig. 2D), inferior olivary (IO; Fig. 2C) and Area II neurons (i.e., vestibulosensitiv). Interestingly, the initial axon trajectories of some neurons in the PN and DO headed toward the CbCom in the same fashion as illustrated for Area II neurons (Fig. 2, B and D), thereby implying that both ipsi- and contralateral hindbrain precerebellar populations reach the Egr through both the vHCom and the CbCom as illustrated for Area II neurons in Figs. 9 and 10 and interpreted in the DISCUSSION.

Axons of contralateral Egr granule cells give rise to axons in the molecular layer constituting the cerebellar crest (Fig. 1F) and along with MO neurons cross in the CbCom (New et al.
1996). The heavy axonal projection observed in the ipsilateral molecular layer (Fig. 1F) shows that Egr granule cells contribute significantly to both lateral line as well as vestibular (i.e., Area II oculomotor-related) simple spike activity of Purkinje cells (Fig. 3Bf) (Beck et al. 2006; Pastor et al. 1997). Based on Area II and horizontal semicircular canal nerve afferent termination patterns after biocytin application (Fig. 2, E and F) the primary location of oculomotor-related granule cells is the anterior and medial Egr as opposed to the caudal and lateral Egr, including the entire medial granule cell division known as pars medialis (pm in Fig. 1D) (Bass 1982). In particular, the termination pattern of the horizontal canal nerve was quite limited in the Egr (Fig. 2F), not extending into the more medial vestibulolateral lobe and corpus cerebelli granule cell layers like Area II neurons (Fig. 2E). The VL and corpus cerebelli granule cell layers are visibly separated by a shared axonal pathway containing the axons of IO neurons (Fig. 2, E and F: curved red arrows) and Purkinje cells that project to the octaval nuclei (Fig. 1D; curved black arrow).

Hindbrain location of Area II

Based on the spatial distribution of retrogradely labeled neurons in Area II, the longitudinal extent of this hindbrain nucleus is equivalent to that of the IO (Fig. 1, B and F–I). However, in contrast to Area II, IO neurons were never retrogradely labeled from Egr biocytin applications (Figs. 1, H and I and 2C). This finding shows that IO neurons do not have Egr collaterals and corroborates the location of the IO axonal pathway in VL between Egr and LC (Fig. 2E, red arrows). Apart from the extensive labeling of neurons in bilateral Area II, other brain stem sources included ventral reticular nuclei (Figs. 1H and 2A), dorsal hindbrain (at level of Fig. 1H), and, in larger numbers, vestibular/oculomotor-related neurons in the ventral part of the descending octaval nucleus (Figs. 1F and 2D) (Green et al. 1997).

Area II neurons were predominant both in number and clustered appearance as shown in the high-magnification section in Fig. 2, A and C. In several consecutive 75-μm sections like that shown in Fig. 2A, >30 Area II neurons per section were encountered over several sections. The number of labeled Area II neurons observed in one section alone is more than the total found in any other hindbrain nucleus related to horizontal semicircular canal afferent terminals. Area II appears as a nearly continuous, but slightly more coarse, layer (Beck et al. 2006; Pastor et al. 1997). Based on Area II and horizontal semicircular canal nerve afferent termination patterns after biocytin application (Fig. 2, E and F) the primary location of oculomotor-related granule cells is the anterior and medial Egr as opposed to the caudal and lateral Egr, including the entire medial granule cell division known as pars medialis (pm in Fig. 1D) (Bass 1982). In particular, the termination pattern of the horizontal canal nerve was quite limited in the Egr (Fig. 2F), not extending into the more medial vestibulolateral lobe and corpus cerebelli granule cell layers like Area II neurons (Fig. 2E). The VL and corpus cerebelli granule cell layers are visibly separated by a shared axonal pathway containing the axons of IO neurons (Fig. 2, E and F: curved red arrows) and Purkinje cells that project to the octaval nuclei (Fig. 1D; curved black arrow).

Efferent organization of the vestibulolateral lobe

An important issue concerning the teleost cerebellum has been distinguishing both the anatomy and the physiology of presumed Purkinje cells from eurydendroid cells that were thought to be displaced cerebellar nuclei neurons responsible for the cerebellar efferent output (Kotchabhakdi 1976b; Murakami and Morita 1987). In 10 experiments, 12 Purkinje cells were identified in the caudal lobe based on antidromic activation and orthodromic mossy and climbing fiber responses (Fig. 3, Ba–Bd) and 8 were well labeled intracellularly (Fig. 3, A and D). The two Purkinje cells illustrated exhibited similar morphology with respect to spines (Fig. 3, C and F), axon hillock (Fig. 3E), and recurrent collaterals (Fig. 3, D and E) within the vestibulolateral lobe, including axons that entered the octaval nuclei (Fig. 3, A and D), giving rise to terminal boutons in the most rostral descending octaval vestibular subdivision from where the ascending tract of Deiters originates (DT, Fig. 3D) or in the caudal and ventral subdivision labeled herein as DO (Fig. 3D; see also Fig. 1B).

The Purkinje cell shown in sagittal sections at low (Fig. 3A) and high (Fig. 3C) magnification displayed a ventrally directed dendritic tree, whereas that shown in the coronal section (Fig. 3D) exhibited a dorsally directed dendritic tree. This latter cell, however, also showed a parasagittal planar direction of Purkinje cell dendrites (as in Fig. 3A). This “inverted” morphology was clearly reflected in the extracellular records of SS and CS activity shown in Fig. 3, Ba and Bb. Antidromic activation of Purkinje cells after vestibular stimulation at the level of the DO nucleus (Ves in Fig. 3Bc) exhibited initial segment/somadendritic (IS-SD) inflection after double-shock stimulation (Fig. 3Bc). Spontaneously recorded SS and CS activity was also frequently recorded with similar durations in both anesthetized and alert goldfish (Pastor et al. 1994a, 1997). Recurrent axon collaterals originating from the Purkinje cell shown in coronal view (Fig. 3D, arrows) arborized throughout the Purkinje cell layer located adjacent to the Egr. The SD, IS, and recurrent terminal (ter) boutons are illustrated in Fig. 3E (arrows) as well as the high density of dendritic spines (Fig. 3F) observed in both Purkinje cells. By contrast, several eurydendroid neurons (i.e., displaced cerebellar nuclei neurons) were intracellularly recorded and labeled in the ventral corpus cerebelli (H. Suwa and R. Baker, unpublished observations), although their morphological profiles differed from Purkinje cells and closely

![FIG. 3. Intracellular identification of Purkinje cells in the goldfish vestibulolateral lobe. A–C: biocytin-injected Purkinje cell shown at low (A) and high (C) magnification in sagittal sections 0.5 mm from the midline. Extracellular spontaneous simple spike (SS) and complex spike (CS) activities of the Purkinje cell are shown in Ba and Bb, respectively. Intracellular antidromic activation of the Purkinje cell after electrical stimulation at the level of the vestibular nucleus (Ves) is shown in Bc and the superimposed spontaneously recorded CS in Bd. Antidromic response is shown at an expanded timescale in Be after double-shock stimulation to illustrate the initial segment/somadendritic inflection (IS-SD). A spontaneously recorded sequence of SS and CS is illustrated in Bf. Timescales and amplitudes are indicated in each set of records. C: ventral dendritic (dend) ramification into the molecular layer (mol) and Purkinje cell axon (ax) is shown with respect to the Egr and corpus cerebelli granule cell layer (gr). D–F: antidromically activated Purkinje cell shown in a coronal section at the level indicated by the yellow dashed line in Fig. 1A. Soma, dendrites (dend), and axon trajectory (ax, arrows) including termination (ter) in the vestibular subgroup of the ascending tract of Deiters (DT) are indicated in D. Soma, initial segment (IS), recurrent terminal boutons (ter, arrowheads), and dendritic spines (sp, arrowheads) on a Purkinje cell secondary dendritic shaft are illustrated at higher magnification in E and F, respectively. Abbreviations as in Fig. 1.](http://jn.physiology.org/7337004)
matched those of cerebellar neurons recently described in goldfish (Ikenaga et al. 2005).

Electrophysiology of Area II neurons

A cerebellar projection from Area II neurons was identified by the presence of antidromic action potentials evoked after electrical stimulation of the Egr (16 of 19 recorded neurons). Three neurons were antidromically activated from the ipsilateral Egr, four from the contralateral Egr, and nine from both sides of the cerebellum. The latencies of antidromic spikes evoked from the ipsi- (Fig. 4A) and contralateral Egr (Fig. 4B) were similar and averaged 0.9 ± 0.2 ms \((P = 0.24; n = 25)\). The short and invariant latency as well as the absence of underlying synaptic potentials (Fig. 4B, inset) identified these spikes as antidromic and excluded an orthodromic origin. Based on these data, 56% (9/16 Area II neurons) exhibited a bilateral Egr projection, 19% (3/16 neurons) an ipsilateral projection, and 25% (4/16 neurons) a contralateral projection. However, the number of bilateral Egr projecting neurons was likely underestimated because the stimulation electrodes were quite superficially placed in the majority of the experiments to preserve Egr morphology.

The vestibular input to Area II neurons was characterized by electrical single-shock activation of the ipsi- and contralateral horizontal semicircular canal nerves. The responses consisted of a crossed excitation (Fig. 4C) and an uncrossed inhibition (Fig. 4D) after separate vestibular stimulation of either side. Electrical stimulation of the contralateral nerve (cVes) evoked excitatory postsynaptic potentials (EPSPs) (Fig. 4C) with latencies that ranged from 1.5 to 2.3 ms in different cells with an average of 1.9 ± 0.4 ms \((n = 19)\). Comparison with the latencies of vestibular inputs to oculomotor and abducens motoneurons recorded in goldfish after electrical stimulation of the vestibular nerve (Graf et al. 1997; Pastor et al. 1992) suggested a disynaptic connection between vestibular nerve afferents and Area II neurons. EPSP amplitudes were variable between different cells, but usually ranged between 1 and 6 mV. In most cases, disynaptic EPSPs were complemented by oligosynaptic EPSPs as seen in the averaged trace in Fig. 4C and at higher stimulus intensities by single and multiple action potentials (not illustrated).

Electrical stimulation of the ipsilateral horizontal canal nerve (iVes) evoked inhibitory postsynaptic potentials (IPSPs) (Fig. 4D) with a latency that ranged from 1.6 to 2.4 ms in different cells with a mean of 2.0 ± 0.4 ms \((n = 19)\), suggesting a disynaptic connection between ipsilateral vestibular nerve afferents and Area II neurons. IPSP amplitudes were as variable as those of the EPSPs between different cells and ranged between 1.5 and 8 mV. In about half of the recorded neurons \((n = 10)\), the IPSP was preceded by a short-latency depolarization with an amplitude never >1 mV whose voltage time course was truncated by IPSP onset (see Fig. 4C, inset). Membrane depolarization did not affect this short-latency EPSP, indicating that it was not a reversed IPSP. The average latency of this EPSP component, 1.2 ± 0.2 ms \((n = 10)\), suggested a monosynaptic excitation of Area II neurons by ipsilateral horizontal canal afferent fibers as summarized in Fig. 10.

Morphology of Area II neurons

Seven of 25 successfully labeled Area II neurons were selected for illustration. In general, the cell bodies of Area II neurons formed a relatively homogeneous population in the ventrolateral hindbrain between the facial lobe and spinal cord (Figs. 1, G–I, 2A, and 5–9). These neurons were somewhat round or spindle-shaped and small to medium sized with a
diameter ranging between 10 and 20 μm (Figs. 5–9). Organization of the dendritic tree for the Area II neuron electrophysiologically characterized in Fig. 4 was studied in further detail after intracellular staining with neurobiotin and camera lucida reconstruction (Fig. 5). The dendritic pattern was typical for the majority of labeled Area II neurons because two main dendritic fields, rostromedial (rm) and caudolateral (cl), extended horizontally just above the pial surface of the brain stem (Fig. 5). The primary dendrites rapidly branched into second- and higher-order dendritic branches that spread out about 0.3–0.5 mm in both rostral and caudal directions relative to the location of the cell body. The mediolateral extension ranged from 0.1 to 0.3 mm medial and 0.4 mm lateral with respect to the cell body. Thus the dendritic tree appeared to offer two major sites for synaptic input: a rostromedial and a caudolateral location with respect to the cell body as illustrated for the Area II neuron reconstructed in Figs. 5–7. Some smaller dendrites located near the cell body. Thus the dendritic tree appeared to offer two major sites for synaptic input: a rostromedial and a caudolateral location with respect to the cell body as illustrated for the Area II neurons reconstructed in Figs. 5–7. Some smaller dendrites extended laterally toward the ventral part of the DO and the posterior octaval (PO) nucleus where horizontal canal–related second-order vestibular neurons were previously recorded (Fig. 2D) (Green et al. 1997). In addition, the soma and the major part of the caudal and lateral dendritic field overlapped largely with the termination area of ipsilateral horizontal semicircular canal and utricular afferents, providing a likely structural correlate for the monosynaptic EPSP shown in Fig. 4D (H. Straka, E. Gillano, and R. Baker, unpublished observations). Most Area II neurons had only a few scattered spines on second- and higher-order dendritic branches compared with the abundance of spines on Purkinje cells (Fig. 3F).

In 17/25 Area II neurons, labeling was sufficient to allow a complete reconstruction of the axonal trajectory in the brain stem and cerebellum. In 15/17 neurons (roughly 85%), the axons projected ipsilaterally toward the cerebellum as shown in Fig. 5, whereas the axons of 2/17 neurons (roughly 15%) crossed within the hindbrain at the level of the cell body and entered the cerebellum at the contralateral side (Fig. 9). Typically, the axons of neurons projecting ipsilaterally to the cerebellum (Figs. 5–8) coursed laterally just above and along the pial surface of the brain stem without collaterals in the ipsilateral Area II. Axonal trajectories then headed dorsally and rostrally, passing through the DO and TAN, giving off either a few (Fig. 5) or a large number of axon collaterals in these two octaval subnuclei (Figs. 6 and 7). In 6/17 neurons, axon collaterals were observed in the caudal part of AO where numerous vestibular commissural neurons are located (Straka et al. 2002). No axon collaterals from Area II neurons were seen to project toward and/or terminate within either the abducens (VI) motor or internuclear nuclei. After entering the cerebellum, the axons branched extensively in the Egr including, in nearly all cases, a few collaterals that projected rostromedially into the granule layers between the vestibulolateral lobe and the corpus cerebelli (Figs. 2E and 5–9). All axon collaterals including those in the vestibulolateral subnuclei were characterized by large terminal boutons along their trajectory (drawings in Figs. 7 and 9). Independent of the density of the termination in the Egr almost all ipsilateral projecting neurons (14/15) sent a single collateral through the cerebellar commissure to arborize in the contralateral Egr as seen in the reconstructed axon trajectory (Fig. 5). In this case, the axon collateralized enough to almost provide a mirror-image pattern on the other side.

Analysis of the projection patterns of the reconstructed neurons showed that 14/17 of the cerebellar-projecting Area II neurons (82%) had a bilateral projection, whereas 18% had either a purely ipsilateral (1/17 neurons; 6%) or a purely contralateral (2/17 neurons; 12%) projection. Combining the results from the antidromic stimulation study with the tracing of the labeled axon to the cerebellum suggests that, minimally, 70% of the Area II neurons exhibit a bilateral cerebellar projection. From the remaining Area II neurons, similar numbers project only to the ipsi- (12%) or contralateral (18%) cerebellum. This proportion of bilateral neurons was likely a better indicator than the view from retrograde labeling (Fig. 1) because terminals from the axon collaterals in CbCom were limited to the medial Egr (Fig. 2E).

Variations in the strength of synaptic connectivity

Although all neurons in Area II exhibited an ipsilateral inhibition and contralateral excitation after electrical stimulation of the horizontal semicircular canals, the variability of the rise time of the synaptic potentials, especially those of the crossed EPSPs, suggests a different strength of synaptic connectivity for different neurons. In some neurons the crossed excitation consisted of a slowly rising EPSP with a relatively small amplitude (Fig. 6A) in contrast to the fast rising EPSP present in the neuron of Fig. 4C. The amplitude and rise time of the uncrossed IPSP (Fig. 4D), however, was similar to that recorded from the neuron shown in Fig. 6B, although a short-latency depolarization like that found in Fig. 4 was absent. The rise times of EPSPs also varied between neurons recorded in the same experiment and at similar resting membrane potentials (>50 mV). Thus it is unlikely that the observed differences in slopes arise from significant differences in conditions between individual preparations, but rather vary between different subpopulations of Area II neurons. In contrast, the gross morphological properties including somadendritic organization and axonal projection pattern of Area II neurons with slow rising EPSPs did not differ systematically from those with fast rising EPSPs.

Synaptic inputs from electrically coupled vestibular neurons

In 4/19 (21%) electrophysiologically characterized Area II neurons, a short-latency depolarization with an amplitude of ±1 mV (Fig. 7A) preceded the chemically mediated disynaptic EPSP after electrical stimulation of the contralateral vestibular nerve. Because the average latency of 1.4 ± 0.2 ms (n = 4) was too short to accommodate two chemical synapses and because first-order vestibular nerve afferent fibers do not cross the midline of the brain stem, the short-latency depolarization must be mediated by second-order vestibular neurons electrically coupled to horizontal canal nerve afferent fibers by gap junctions (Graf et al. 1997). The transynaptic labeling of second-order horizontal canal neurons with an axonal projection to the contralateral Area II after application of biocytin to the horizontal nerve branch (H. Straka and R. Baker, unpublished observation) is compatible with the presence of a short-latency depolarization in some Area II neurons. Stimulation of the ipsilateral vestibular nerve evoked a fast rising IPSP with a disynaptic onset (Fig. 7B) that, however, was not preceded by a short-latency depolarization as observed in Fig. 4C. Both the
FIG. 5. Camera lucida drawing of the precerebellar Area II neuron physiologically characterized in Fig. 4. Reconstruction of the biocytin-filled Area II neuron shows a typical morphology. Note the extensive dendritic arborization within the ventral hindbrain nucleus, an ipsilateral course of the axon (ax) toward the cerebellum, and 2 small collaterals in the (DO) nucleus before branching extensively in the ipsi- (iEgr) and contralateral eminentia granularis (cEgr) of the vestibulolateral lobe (also see overview, top left). Higher-magnification inset: somadendritic field of this neuron subdivided into a rostromedial (rm) and a caudolateral (cl) field. Four photomicrographs show 1) the ventral location of the soma, 2) ascending axon entering the Egr dorsal to AO, 3) the contralateral axon collateral adjacent to the IO/Purkinje cell fascicle, and 4) axon in the CbCom.
FIG. 6. Vestibular input and camera lucida drawing of an Area II neuron with an ipsilateral axon projection to the Egr. A and B: orthodromic activation after single-shock electrical stimulation of the horizontal canal nerves on both sides. Superimposed single sweeps and average (n = 10) of EPSPs and IPSPs after stimulation of the contralateral (cVes, A) and ipsilateral (iVes, B) horizontal canal nerves. Slower rise times of the EPSPs and partial spikes superimposed suggest a different efficacy of the synaptic connections when compared with those shown in Fig. 4. Insets in A and B: onset of the disynaptic EPSPs and IPSPs at an expanded timescale. Dashed lines indicate baseline. Note the absence of a short-latency depolarization preceding the iVes IPSP. Calibration bars in A also apply for B. Reconstruction (overview in top left) indicates the typical Area II somadendritic organization, axonal pathway (ax), and termination in the ipsilateral eminentia granularis (Egr) of the vestibulolateral lobe. This Area II axon more closely followed the lateral pial/ventricular boundary than depicted in the summary. This neuron showed a more extensive "closed field" dendritic arborization than that shown in Fig. 5, but also exhibited a distinct rostromedial (rm) and caudolateral (cl) field. No collateral was found in the CbCom despite intense staining of the ipsilateral termination. Three morphological insets show 1) location of the soma and axon, 2) axon branches and terminals in the tangential nucleus (Tan; white arrows), and 3) axon collaterals near and in the CbCom.
FIG. 7. Orthodromic vestibular input and reconstruction of an Area II neuron with an ipsilateral axon exhibiting extensive collaterals in the vestibular nuclei. A and B: superimposed single sweeps and average \( (n = 10) \) of EPSPs and IPSPs after stimulation of the contra- (cVes, A) and ipsilateral (iVes, B) horizontal canal nerves. Insets: onset of synaptic responses at an expanded timescale including a short-latency cVes depolarization with a monosynaptic latency, suggesting electrotonic coupling by gap junctions of horizontal canal afferents and Area II projecting second-order vestibular neurons (Graf et al. 1997). Dashed lines indicate baseline. Calibration bars in A also apply for B: camera lucida drawing of this neuron with overview in the top left shows a somadendritic morphology and axonal projection (ax) similar to those depicted in Figs. 5 and 6; however, extensive axonal branches were observed within the ipsilateral DO and AO nuclei (arrows show high-magnification reconstruction). Axon collateral crossed in the CbCom; however, arborization in the contralateral Egr was minimal. Higher-magnification drawing of the soma shows the rostromedial (rm) and caudolateral (cl) dendritic fields. Two photomicrographs illustrate 1) axon terminals in the DO and 2) the axon ascending in the Egr with adjacent terminal branches.
short-latency connection between contralateral horizontal canal nerve afferent fibers and Area II neurons and the almost invariantly fast rising IPSP from the ipsilateral nerve (Fig. 7B) suggest a substantial role for the vestibular sensory signals (Beck et al. 2006).

The neuron shown in Fig. 8D was spontaneously active with a moderate resting activity of 30 Hz and recruited by visual flow stimulation to the ipsilateral side. After reconstruction, the morphology and projection pattern were shown to be typical for Area II neurons including two separately directed dendritic fields, one rostromedial and the other caudolateral (Fig. 8F). An ipsilateral axon projected to the Egr with reasonably intense arborization and an axon collateral crossing in the cerebellar commissure to the other Egr (Fig. 8D). In several of the labeled Area II neurons a large number of axon collaterals arborized within the ipsilateral vestibular areas of the AO and DO as shown in Fig. 7. The reconstructed Area II neuron shown in Fig. 8C was not spontaneously active but modulated with visual stimuli to the ipsilateral side (not shown). The comparatively large number of axonal terminations in the vestibular (octaval) subnuclei and the robust synaptic inputs from both horizontal canal nerves by second-order vestibular neurons as summarized in Fig. 10 suggests a strong reciprocal vestibular/Area II connectivity underlying the observed eye velocity signaling (Beck et al. 2006).

**Contralateral projecting Area II neurons**

In 2/17 (roughly 12%) Area II neurons, the axons could be followed to the contralateral cerebellum after crossing to the opposite side in the hindbrain at the level of the nucleus (Fig. 9). The retrograde tracing experiments (Figs. 1 and 2) suggested that Area II neurons with axons decussating within the hindbrain and projecting to the contralateral Egr may be preferentially located in the rostral half of Area II. The axons of these neurons cross either ventrally in a fiber bundle above the pial surface or more dorsally (Fig. 9). After crossing the midline, axons of these Area II neurons joined the fiber tract formed by the Area II neurons that projected ipsilaterally to the vestibulocerebellum. The Area II neuron that crossed dorsally in the caudal brain stem gave off few collaterals in the reticular formation (Fig. 9). Both neurons gave off several collaterals in the DO and also more rostrally in the AO on their rostral trajectory toward the cerebellum (Fig. 9). Axons of both neurons could be followed into the Egr, but in neither case was there any indication of a collateral crossing in the cerebellar commissure. Even though the axonal pathways of the latter two neurons differed from the majority of Area II neurons, ipsilateral horizontal canal nerve stimulation evoked disynaptic IPSPs and the contralateral horizontal canal, disynaptic EPSPs (not illustrated). Thus irrespective of the precerebellar Area II neuron axonal projection pattern (i.e., uncrossed or crossed Egr terminations) a strong reciprocal head velocity signal would be the expected common denominator (Beck et al. 2006).

**DISCUSSION**

**Summary**

The general goal of this study was to establish the morphological organization of the goldfish vestibulocerebellar circuits by specifying the major brain stem mossy fiber projections to granule cells and to directly document Purkinje cell axonal projections to the vestibular nuclei. The primary objective was to characterize the morphology and physiology of the precerebellar hindbrain neurons originally delineated as Area II (Pastor et al. 1994b). Our study showed that every Area II neuron exhibited a disynaptic IPSP and EPSP after selective activation of the ipsilateral and contralateral horizontal canal nerves, a finding clearly correlated with the behavioral signal.
of contralateral head and ipsilateral eye velocity (H1E1) (Beck et al. 2006). Intracellular injection of biocytin in Area II neurons revealed the absence of axon collaterals within or between the bilateral Area II nuclei and a modest but uniform projection to the vestibular subnuclei, in particular to the DO. By contrast, a robust termination pattern was found in the anterior Egret of the bilateral vestibulolateral lobes. These findings establish the anatomy of a basic hindbrain cerebellar circuit (Fig. 10) that, interpreted in the context of neuronal signal sensitivity (Beck et al. 2006), provides an outstanding precerebellar model system to study oculomotor performance and plasticity (Bassett et al. 2005; Beck et al. 2001; Debowy and Baker 2004; Marsh and Baker 1997; Pastor et al. 1997).

Nomenclature of the precerebellar hindbrain neurons

The origin, role, and possible homology of this particular group of hindbrain precerebellar neurons in the goldfish with other vertebrates constitute an outstanding question. Both Area I and Area II (Pastor et al. 1994b) were proposed to be functionally analogous and perhaps homologous to the mammalian prepositus hypoglossi nucleus (McCrea and Baker 1985b). This extrapolation seemed reasonable based on the fact that both subgroups in the goldfish displayed disynaptic EPSPs and IPSPs like those of cat prepositus neurons (Baker and Berthoz 1975) and appeared to be functionally equivalent to the burst/tonic H1E1 cell types observed in the both the cat and primate prepositus hypoglossi nucleus (Lopez-Barneo et al. 1982; McFarland and Fuchs 1992). Nevertheless, the embryological hindbrain neuromeric organization in goldfish larva (Suwa et al. 1996) together with the location of these nuclei in the adult goldfish argue against a strict interpretation of homology.

The Area I neurons (eye position) in the goldfish are clearly located in the central reticular nuclei (Fig. 2C), which is derived from the embryonic basal neural epithelium (Baker and Gilland 1996). By contrast, the Area II neurons are located between the descending octaval and inferior olivary nucleus in the goldfish, both of which are derived from the alar neuroepithelium (Diaz et al. 1998; Marin and Puelles 1995). These precerebellar cells likely migrate ventrally along the pial surface, as do the adjacent inferior olivary neurons (Fig. 2C), and both subgroups are eventually located in the ventral brain stem as are the goldfish abducens motoneurons and internuclear neurons (Pastor et al. 1991). In that regard, Area I (but not Area II) neurons project exclusively to the individual abducens subnuclei (Aksay et al. 2000); unlike in mammals, however, they do not project to either the vestibular nucleus, cerebellum, or the rostral midbrain including the superior colliculus (McCrea and Horn 2005).

In summary, it is possible that only one of the two groups of goldfish neurons (Area I or Area II) was selected to form a functionally analogous (not homologous) nucleus like the mammalian prepositus hypoglossi. More likely, however, the two groups of goldfish neurons have persisted, intermingled and together are located dorsally and caudally to the abducens nucleus in mammals (McCrea and Baker 1985a). Thus the most parsimonious organization in more derived vertebrates is a heterogeneous mixture of the two subgroups that migrated together and lie in what appears to be a continuous dorsal hindbrain column called the prepositus hypoglossi nucleus. In mammals, the functional role of an Area II–like prepositus...
neuron has been either complemented or and superseded by the pontine precerebellar pathways containing both visual and motor signals (Baker and Gilland 1996; Takeichi et al. 2005). From the perspective of an experimental model, the exceptional separation of eye position and eye velocity signals into two separate hindbrain nuclei in goldfish is clearly advantageous because it allows well-controlled perturbations of the precerebellar projection to the vestibulocerebellum.

**Organization of the vestibulolateral lobe of the teleost cerebellum**

The form and size of the cerebellum vary immensely among various teleost species and both the morphology and the function have long been based on a division into three regions (Bass 1982). Both the valvula and vestibulolateral lobe forming the most rostral and posterior portions of the cerebellum (Fig. 1B) are intimately related to the lateral line and vestibuloacoustic function (Larsell 1967). The early anatomical work divided the vestibulolateral lobe into the eminentia granularis and the lobus caudalis, which is somewhat confusing with respect to function because the granule cells form an integral part of the hypothesized cerebellar unit (mosaic fiber–Purkinje pathway). One explanation for the division is that the Egr forms visible bulges along the caudal lateral lobe of the cerebellum and also medially borders the ventricle, which in many teleost species exhibits a molecular layer continuous with that of the cristae cerebellaris (ML in Fig. 1, many teleost species exhibits a molecular layer continuous with that of the cristae cerebellaris (ML in Fig. 1, B and F), overlying the principal lateral line nuclei (MO; Fig. 1, B and F). This caudal cerebellar region was originally termed the “auricle,” a reference more appropriate for the characteristic lateral cerebellar folia described in amphibians (Larsell 1967). A lateral granule cell zone termed “the auriculi cerebelli” and a more medial zone called “pars medialis” along with a molecular zone including larger cells were envisioned to be interconnected by an “interauricular” band (Fig. 1D, SE) called the transverse plate. After the vestibulolateral lobe of teleosts was separated into a more structurally simple Egr and a lobus caudalis (Nieuwenhuys and Nicholson 1969) it became synonymous with Larsell’s transverse plate that also included the caudal and medial subdivisions of the Egr, which as suggested in this study, is not a significant oculomotor-related part of the Egr. This conclusion is based on the termination pattern of precerebellar hindbrain neurons in the Egr (Figs. 2E and 5–9) and on directly visualizing the “interauricular” pathway connecting the bilateral Egr (Fig. 1E).

Our results have identified the rostral Egr as a specific site for mossy fiber afferents related to horizontal oculomotor behavior, which in turn revealed a large hindbrain precerebellar nucleus providing an estimated 75% of the vestibular oculomotor signaling to the cerebellum (Beck et al. 2006). This observed structural arrangement further suggests that the so-called pontine nucleus (Finger 1978a), identified after Egr biocytin labeling (Figs. 1, C and D and 2B) and located at the level of AO, may be the functional counterpart of Area II for vertical eye movements. If so, hindbrain precerebellar pathways might have been evolved in a parallel, symmetrical fashion for all eye movement performance and plasticity involving the vestibulolateral lobe.

The Egr vestibular/lateral line overlap is attested to by the intermingling of lateral line (i.e., water displacement) and eye movement sensitivity in separate Purkinje cells (Pastor et al. 1997). In addition, placement of tracer into Egr always contained labeled axons and cells signifying a mechanosensitive mossy fiber termination and function. These observations indicate that a morphological boundary cannot be distinguished between the lobus caudalis and the cerebellar crest (the presumed lateral line lobe). Based on structure alone, it would be difficult to recognize the physiologically related granular and molecular zones in the vestibulolateral lobe, the valvula, and the corpus cerebelli, but by contrast they can be better distinguished by the mossy fiber afferent projections of the individual Area II neurons (Figs. 5–9).

**Vestibulolateral lobe neurons and connectivity**

Not only is the cerebellum of teleosts unique with respect to the apparent absence of well-delineated deep cerebellar nuclei, but also the variation between species is considerable, particularly in electroreceptive teleosts (Nieuwenhuys and Nicholson 1969). The efferent output of the caudal lobe has been shown in this study to consist of largely, if not exclusively, Purkinje cells and not “eurydendroid cells” (Fig. 3). This term originated with Pouwels (1978a,b) who, in an excellent series of papers on the development of the corpus cerebelli of the trout, presented a wiring diagram of the cerebellar input–output organization formed by mossy fibers, granule cells, and Purkinje cells that also included the eurydendroid cells (Pouwels 1978b). The density of the Purkinje cell inhibitory synaptic arrangements on the eurydendroid cells was striking, whereas by contrast the parallel fibers were observed to contact both Purkinje and eurydendroid cells. In spite of this arrangement, the cerebellar modular organization in teleosts was always envisioned to be the same as that in mammals, even including all the inhibitory interneurons such as Golgi cells contacted by the mossy fibers, establishing a local negative feedback system within the cerebellar cortex (Pouwels 1978a). For example, in the present study, Purkinje cell axon collaterals were observed in the molecular layer (Fig. 3E) where they likely inhibit Golgi cells and other Purkinje cells. In conjunction with the demonstration that Purkinje cells terminate on vestibular neurons in the octaval nuclei, we conclude that, except for the noticeable lack of basket cells around Purkinje cell somata (i.e., like reptiles), the basic cerebellar organization for the teleost oculomotor system is remarkably similar throughout all vertebrates. Based on more recent anatomical and molecular labeling, eurydendroid cells are suggested to be the primary, likely only, output of the corpus of the cerebellum (Ikenaga et al. 2005) and as such are equivalent to mammalian deep cerebellar nucleus neurons as is the vestibular nuclei for Purkinje cells in the vestibulolateral lobe.

**Morphological and physiological identification of vestibulolateral lobe Purkinje cells**

The best and most current study on the morphology and distribution of projection neurons in the caudal lobe of the cerebellum in a teleost distinguished two types of projection neurons called A and B (Murakami and Morita 1987). Type A neurons were found to be specific for the caudal lobe; however, these neurons were considered to be eurydendroid neurons targeting the oculomotor complex (Fig. 9 in Murakami and
Morita 1987) and not Purkinje cells. This conclusion was based on an expected difference in dendritic spine structure for Purkinje cells (small) and the type A neurons (long) (Fig. 6A in Murakami and Morita 1987). However, given the absence of widespread dendritic ramifications (in contrast to the eurydendroid neurons in the corpus of the cerebellum), type A neurons are more likely to be the Purkinje cells electrophysiologically identified in this study.

An intracellular analysis of putative goldfish Purkinje/eurydendroid neurons suggested that vestibular and lateral line input was largely restricted to the caudal lobe (VL) and included ventral portions of the corpus, whereas visual input appeared to be ubiquitous throughout the cerebellum (Kotchabakhdi 1976a,b). This work was the first to document mossy and climbing fiber responses in antidromically identified Purkinje cells that, unfortunately, were interpreted as efferents to hair cells based on electrical stimulation of the vestibular nerve. Electrical stimulation of the VIIIth nerve near the brain stem (Fig. 1C) could also activate Purkinje cells through current spread; however, peripheral electrical stimulation of the vestibular nerves near the ampulla of the semicircular canal did not produce antidromic activation, especially at thresholds producing the vestibular disynaptic EPSPs and IPSPs in the precerebellar hindbrain neurons (Fig. 4). Climbing fiber activation at extremely high thresholds was consistent with the small inferior olivary axons as well as their medial trajectory paralleling Purkinje cell axons (Figs. 2, E and F and 3D).

The spine-studded secondary and tertiary dendritic trees of every antidromically identified Purkinje cell along with the sparsely spined structure of the main dendritic shaft together with the characteristic Purkinje cell initial segment followed by extensive axon collaterals ramifying within the molecular layer constitute criteria that now demonstrate homology of goldfish Purkinje cells with those in other vertebrates. Not only the clear contrast between mossy fiber action potentials and the complex spikes (1 vs. 20 ms in duration) but also the frequent observation of complex spikes at the pause in mossy fiber-driven simple spike discharge (Fig. 3Bf) reinforce a strong similarity in features that are classically described in other vertebrates.

Of particular note is that within the molecular layer of the caudal lobe, the dendritic tree of goldfish Purkinje cells is organized principally in the sagittal plane, which is indicative of parallel fiber axon trajectory; however, like in amphibians (Nieuwenhuys and Nicholson 1969) the branching pattern is more collapsed as opposed to planar in nature (Fig. 3A). Correspondingly, we have observed that it is only infrequently that extracellular SS and CS exhibit comparable (overlapping) extracellular dipole fields from mossy and climbing fiber activation (Pastor et al. 1997). Nevertheless, we conclude that the caudal lobe in the goldfish likely contains only Purkinje cells whose signaling sensitivity is remarkably consistent with that described for the precerebellar hindbrain Area II neurons (Beck et al. 2006).

**Correlation of morphological and electrophysiological properties of Area II**

Routine intracellular scans of the entire nucleus, from the rostral to caudal end, showed the synaptic potential pattern to be the same—even though considerable variations existed in rise time, amplitude, and duration—that in each case appeared to be independent of short-circuiting artifacts from in vivo sharp electrodes. Perhaps this variation in electrophysiological characteristics is correlated with the observed somadendritic variation seen in these neurons as illustrated in the composite drawings shown in Figs. 5–9. Many reticular neurons surrounding the Area II nucleus (Fig. 2A) also received vestibular inputs, but almost exclusively EPSPs and infrequently IPSPs. Thus the general conclusion is that Area II neurons are the responsible synaptic input to granule cells in the vestibulocerebellum, yet signal distribution within the molecular layer is unknown as is continuity of Area II structure/function to Purkinje cells. Interestingly, the ipsilateral cerebellar projection implies monocularity in the mossy fiber projection on the granule cells, yet the widespread distribution of a common eye velocity signal (H,E) bilaterally in the Egr precludes any conclusion regarding Purkinje cell firing pattern in terms of the directionality of eye movements.

Every precerebellar neuron was antidromically activated from the ipsi- and/or contralateral cerebellum. Thus the prediction would be that retrograde tracing after ipsilateral Egr bicystein application would label a nearly equal number of Area II neurons on either side. However, in all cases ipsilateral Area II neurons predominated, suggesting that not all crossed the cerebellar commissure. This delineation was corroborated by the single-cell injections and reconstructions showing that the vast majority of Area II neurons were ipsilateral (roughly 85%) and only very few were contralateral (roughly 15%), with nearly 65% exhibiting bilateral termination in the vestibulolateral lobe. Nevertheless, both ipsi- and contralateral Area II neurons appeared to exhibit modest to robust synaptic connection with vestibular neurons, suggesting a feedback circuitry for vestibular signaling, presumably arising from the horizontal semicircular canals. In this regard, the absence of axon collaterals within or between the precerebellar Area II nuclei precludes commissural feedback interaction, but does raise the issue of a structural basis for the observed velocity storage neural integration (Beck et al. 2006). Because eye velocity build-up is robust in the goldfish, especially in the absence of the cerebellum (Marsh and Baker 1997), the behaviors must be intrinsic to either vestibular or Area II neurons. Second-order vestibulocerebellar neurons terminating in the abducens nucleus subgroups appear to be one of the major inputs to Area II neurons and presumably vice versa (Figs. 5–9), and thus eye velocity integration is more easily conceived as a circuit phenomenon.

Based on both the electrophysiological and the morphological results it is likely that Area II contains no local interneurons. Thus within the brain stem, Area II neurons are concluded to project principally to the DO of the octaval complex, largely ipsilateral as opposed to contralateral, without direct projections to either the abducens motoneurons, internuclear, or Area I eye velocity-to-position integrator neurons (Fig. 10). Given that all mossy fiber inputs to the cerebellum are excitatory, it is inevitable to conclude that all Area II neurons are excitatory as well. Every Area II neuron identified electrophysiologically along the rostral/caudal extent of the nucleus exhibited the disynaptic EPSPs and IPSPs from the horizontal canal nerve and some a small presumably monosynaptic EPSP (Fig. 5). Quite remarkably, this is the exact sequence of synaptic potentials found in abducens motoneurons in both...
goldfish and mammals, clearly indicating an evolutionarily conserved synaptic pattern in this particular type of neuron (Graf et al. 1997). This structural attribute, more than any other feature, portends the possible role of these neurons in the goldfish, but also in the mammalian prepositus hypoglossi nucleus. Undoubtedly, Area II neurons act as a summing junction for vestibular and visual sensory signals and as such are chiefly responsible for the eye velocity sensitivity of Purkinje cells in the goldfish vestibulolateral lobe (Beck et al. 2006; Pastor et al. 1997).

A C K N O W L E D G M E N T S

We thank D. Chu for excellent technical assistance.

G R A N T S

This research was supported by National Institutes of Health grants to R. Baker and a scholarship from the Max-Kade Foundation New York to H. Straka.

R E F E R E N C E S


