Physiology of Morphologically Identified Cells in the Posterior Caudal Lobe of the Mormyrid Cerebellum

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Submitted 3 May 2007; accepted in final form 28 June 2007

Zhang Y, Han VZ. Physiology of morphologically identified cells in the posterior caudal lobe of the mormyrid cerebellum. J Neurophysiol 98: 1297–1308, 2007. First published July 5, 2007; doi:10.1152/jn.00502.2007. The cerebellum of the mormyrid fish consists of three major divisions: the valvula, the central lobes, and the caudal lobes. Several studies have focused on the central lobes and the valvula, but little is known about the caudal lobes. The mormyrid caudal lobe includes anterior and posterior components. The anterior caudal lobe is associated with the lateral line and eighth nerve end organs, whereas the posterior caudal lobe is associated with the electrosensory system. The present study examines the physiology and pharmacology of morphologically identified Purkinje cells and efferent cells in an in vitro slice preparation of the posterior caudal lobe. We found that the Purkinje cells in the posterior caudal lobe can be classified into three subtypes based on both their morphology and on their physiological responses to intracellular current injection and to synaptic inputs from parallel fibers and climbing fibers. Similarities and differences between the physiology of the caudal lobe and that of other regions of the mormyrid cerebellum and the mammalian cerebellum are discussed.

INTRODUCTION

The cerebellum of mormyrid electric fish is well known for its extraordinary size and for the crystalline regularity of its histological structure. The morphology of this unusual cerebellum has been studied extensively (Meek and Nieuwenhuys 1991; Nieuwenhuys and Nicholson 1969a,b), but much less is known about its physiology. The size and regularity of the mormyrid cerebellum as well as other morphological features of the mormyrid cerebellum suggest that knowledge of its cellular physiology could contribute to an understanding of cerebellar circuitry in general.

The cerebellum of the mormyrid fish and other teleosts consists of three major divisions: the valvula, the central lobes, and the caudal lobes (Meek 1998; Nieuwenhuys and Nicholson 1969a). The central lobes are probably homologous to the corpus cerebelli and vestibular cerebellum of mammals; the caudal lobe is likely similar to the vestibulocerebellum of mammals, but the valvula cerebelli is present only in ray-finned fishes (Bell and Szabo 1986; Finger 1982). Recent studies have examined the physiology of the mormyrid central lobes (Han and Bell 2003) and valvula (Zhang et al. 2006), but there have been no physiological studies of the caudal lobe.

The caudal lobe is best developed in electroreceptive teleosts, the best-known examples are weakly electric mormyrid and gymnotid fish (Finger 1982). In both mormyrids and gymnotids, the caudal lobe is closely associated with the first stage of electroreceptive processing, the electroreceptive lobe (ELL). The ELL is a cerebellum-like structure with a molecular layer composed of parallel fibers arising from an overlying granule cell mass known as the eminentia granularis. In both mormyrids and gymnotids, the same granule cell masses provide parallel fiber input to both the ELL and to the caudal lobe (Bell and Szabo 1986; Meek 1998; Sas and Maler 1987).

Functionally, it has been demonstrated that the caudal lobe of Gymnotid plays an important role in modulating sensory processing in the electroreceptive system (Bastian 1986; Bastian et al. 2004). The caudal lobe of mormyrid is separated into anterior and posterior portions. Three granule cell masses, the anterior, medial, and posterior eminentia granularis provide parallel fibers to the lobes (Bell and Szabo 1986; Finger 1982). Functionally, the anterior caudal lobe is associated with sensory inputs mediated by the mechanical lateral line and eighth nerve endorgans, whereas the posterior caudal lobe is associated with the electroreceptive system (Bell 1986; Bell et al. 1992; Campbell and Bell 2003; Campbell et al. 2007). The posterior eminentia granularis provides parallel fibers to both the caudal lobe of the cerebellum and to the electroreceptive lobe, the latter being the first stage in the central processing of electroreceptive information. Moreover, the efferent neurons from the caudal lobe project to electroreceptive structures in the midbrain (Bell et al. 1981), and in vivo recording show that activities of the posterior caudal lobe are strongly affected by electroreceptive input and by corollary discharge signals associated with the motor command that drives the electric organ to discharge (Bell et al. 1992; Campbell and Bell 2003).

The caudal lobe of the cerebellum in weakly electric fish is interesting because of its close relationship to the electroreceptive system and because it receives the same parallel fiber input as the ELL. These are important features suggesting that the caudal lobe of weakly electric fish may provide a clear example of a cerebellum that is involved in sensory information processing. In addition, electroreceptive input can be experimentally controlled in a very precise manner (Bastian et al. 2004; Bell et al. 1993). Therefore we believe that the caudal lobe of mormyrid and other weakly electric fish is a suitable model for physiological studies of cerebellar function in general.

The cellular morphology of the posterior caudal lobe was recently examined in detail by Campbell et al. (2007), showing...
that the local circuitry in the posterior caudal lobe differs from that of the central lobes as well as that of the valvula (Meek 1998; Nieuwenhuys and Nicholson 1969a) in lacking both clear laminar organization and the palisade pattern of Purkinje cell dendrites. These striking differences in local circuitry between the caudal lobe and other cerebellar structures of the mormyrid cerebellum suggest that the posterior caudal lobe may function differently.

The present study has examined the basic physiology and pharmacology of cells in the posterior caudal lobe, we recorded intracellularly the responses of posterior caudal lobe cells to extracellular stimulation of parallel fibers and climbing fibers and to intracellular current injection into the soma in an in vitro slice preparation. We also examined the basic pharmacology of these responses. Finally, we identified the morphology of the recorded cells by injecting biocytin or neurobiotin into the recorded cells, to correlate their physiology, pharmacology, and morphology, to establish the functional circuitry for this portion of the mormyrid cerebellum.

Our results show that the basic features of cell physiology in the central lobes (Han and Bell 2003) and the valvula (Zhang et al. 2006) are also present in the cells of the posterior caudal lobe. Some distinctive features, however, were also observed, including different physiological and morphological subtypes of Purkinje cells, an apparent lack of all-or-none climbing fiber input onto some subtypes of Purkinje cells, and minimal disynaptic inhibition evoked by parallel fiber stimulation.

METHODS

General

All experiments were carried out in in vitro slices from the cerebellum of mormyrid fish of the species Gnathonemus petersii. A total of 43 fish were used for these experiments, ranging in length from 6 to 15 cm. Fish were obtained from local wholesale dealers and were housed and handled according to national and institutional guidelines. All experiments were approved by the Institutional Animal Care and Use Committee of Oregon Health Science University (IACUC No. 0715). Both parasagittal and transverse slices were used. Intracellular recordings were made using whole cell patch electrodes in a submerged chamber under visual control. Stimulating electrodes were placed in the area near the recorded cell to activate climbing fibers and more distally to activate parallel fibers.

Slice preparation

The fish were deeply anesthetized with tricaine methane sulfonate (MS-222) at a concentration of 100 mg/l. The skull was opened, and the brain was fully exposed and irrigated with ice-cold, artificial cerebrospinal fluid (ACSF; for composition, see following text). The whole brain was quickly removed and left in ice-cold ACSF for ~1 min to harden the tissue. The parasagittal and transverse slices were prepared in the following way. The trimmed brain blocks containing the caudal lobe were glued to a vibratome (Leica, TV 1100S) plate. The brain blocks were supported during cutting by a U-shaped wall of gelatin (12% in saline) that was glued behind the brain blocks. Liquid gelatin (same concentration in liquid form at ~30°C) was poured between the gelatin wall and the surface of the brain to provide further support. The cutting chamber was filled with ice-cold ACSF, equilibrated with 95% O2-5% CO2 during slicing. At this point, the ACSF contained nonselective glutamate receptor blocker kynurenic acid (1 mM) to reduce potential excitotoxic damage (Rossi et al. 2000). The composition of the ACSF was as follows (in mM): 124 NaCl, 2.0 KCl, 1.25 KH2PO4, 24 NaHCO3, 2.6 CaCl2, 1.6 MgSO4.7H20, and 20 glucose (pH 7.2–7.4, osmolarity: 295–305). After cutting (typically at 200 μm) slices were immediately transferred into the ACSF containing 0.5 mM kynurenic acid that was kept in a warm bath (26–28°C) for ≤1 h, then left in normal ACSF (without glutamate blocker) at room temperature until recording.

Individual slices were transferred to a submerged recording chamber for whole cell patch recording. The slices were bathed in a continuous flow of oxygenated normal ACSF at 1–1.5 ml/min during recording.

Recording and stimulation

The posterior caudal lobe could be easily recognized under a microscope in transverse and parasagittal slices. Cells for recordings were randomly selected from different parts of the lobe in the initial stage of the experiments. All recordings were made at room temperature (22–24°C). P-clamp 9 software from Axon Instruments was used for data acquisition and analysis.

The electrodes used for whole cell patch recording had resistances of 4–8 MΩ after being filled with an internal solution that contained 0.5% biocytin or neurobiotin. The composition of the internal solution was as follows (in mM): 130 K gluconate, 5 EGTA, 10 HEPEs, 3 KCl, 2 MgCl2, 4 Na2 ATP, 5 Na2 phosphatethe, and 0.4 Na2 GTP (pH 7.3–7.4, osmolarity: 280–290). Cells were visualized under infrared Nomarski optics using the x 40 water-immersion objective of an upright microscope (Axoskop I, Zeiss). Typically, a gigahm seal was established by a gentle suction and the membrane was ruptured by a small negative pressure or zap (a brief electric pulse generated by the amplifier). Fast and slow capacitances were corrected using the automatic capacitance compensation function of the amplifier before and after the membrane rupture. Series resistance was compensated by ~75% in most cases. The recordings were performed under both voltage and current clamp modes using a Multiclamp 700A amplifier. The holding potential under voltage clamp was ~70 mV unless pointed out.

Recordings were discontinued if the resting membrane potential fell below ~55 mV under current clamp or when the leak current was >100 pA under voltage clamp. The access resistance, cell input resistance and cell capacitance were also periodically automatically monitored by recording software P clamp (Axon Instruments). Recordings were terminated if the access resistance was >35 MΩ.

After the observations of electrophysiology and pharmacology, biocytin or neurobiotin in the recording micropipettes was ejected into the recorded cells by applying tip-positive current pulses (500 ms on, 500 ms off, 0.5–1.0 nA for 10–15 min). Slices were fixed in a solution containing 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer within 30–60 min after the injection of tracer. Slices were kept in the same fixative overnight.

We used pairs of tungsten electrodes or single glass pipettes filled with external solution for stimulation. One member of the tungsten electrodes was placed in the tissue and the other member in the bath. For stimulation with glass pipette, the tip of the pipette was placed in the tissue, and another wire was submerged in the recording chamber bath. Stimulus pulses of 0.1 ms in duration and 10–100 μA intensity with the electrode in the tissue negative against the bath were delivered through a stimulus isolation unit. The stimulating electrode was placed in the molecular layer to activate parallel fibers and in the area near the patched cells to activate climbing fibers (Fig. 2A).

Pharmacological and data analysis

The following pharmacological compounds were used: the glutamate a-amino-3-hydroxyl-5methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6-cyano-7-nitroquinoline-2,3-dione (CNQX, 10–20 μM); glutamate N-methyl-d-aspartate (NMDA) receptor antagonist t(–)2-amino-5-phosphonopentanoic acid (AP5, 35 μM); the GABA A receptor antagonist bicuculline methiodide (50 μM) applied for 5 min; 6-cyano-7-nitroquinoline-2,3-dione (CNQX, 10–20 μM); and glycine (20–200 μM).
antagonists bicineuline methiodide (30 μM) and picrotoxin (100 μM); the sodium channel blocker tetrodotoxin (TTX, 1 μM); and the calcium channel blocker cadmium chloride (100–200 μM). All of the chemicals were purchased from Sigma.

Quantitative analysis was done by measuring the latency, peak amplitude and decay constant of excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) generated by brief extracellular stimulus pulses to activate parallel fibers and climbing fibers. Paired-pulse interactions were evaluated by delivering pairs of stimuli between 10 and 1,000 ms in some cases and between 30 and 50 ms in most cases. Facilitation and depression were calculated by subtracting responses to single stimulus (control) from responses to paired stimuli, and peak values of control responses were used to normalize the second responses. The details of this procedure are illustrated in Fig. 7. All measurements were carried out using Clampfit (Axon Instruments). Statistical significance was assessed by comparing the parameters among the groups of cells using Origin (OriginLab). Results will be presented as means ± SE.

**Histology**

For morphological identification of cell types, slices, in which cells had been recorded with whole cell patch electrodes, were routinely treated by histology procedures. The detailed procedures were described elsewhere (Han et al. 1999, 2006). Briefly, to visualize the biocytin or neurobiotin in the recorded cells with fluorescent dyes, the slices were incubated in streptavidin-conjugated Alexa fluor 594 or Texas Red (1:200 in 0.1 M phosphate buffer; Molecular Probes), then mounted in the fluorescent mounting medium Vectashield (Vector). In some experiments, slices were incubated in streptavidin-conjugated fluorescent dyes to reveal the complete dendrites and axon arbors.

To visualize the biocytin or neurobiotin in the recorded cells with diaminobenzidine (DAB), slices were resectioned following overnight fixation. The detailed method for revealing biocytin filled cells with the DAB method has been described elsewhere (Han et al. 1999, 2006). Biocytin- or neurobiotin-filled cells revealed with the DAB method were examined using a light microscope and reconstructed using a camera lucida attachment. Biocytin-filled cells revealed with the fluorescent method were examined in a microscope equipped with epifluorescence and in a confocal microscope. Our morphological identification of stained cells in the posterior caudal lobe was based on the recent descriptions of cells by Campbell et al. (2007).

**RESULTS**

**Anatomical overview**

The posterior caudal lobe in the mormyrid extends along either side of the midline and is flanked at its lateral margins by the eminentia granuljaris posterior (EGp). It is located dorsal to the electrostimulatory lobe and caudal to the central lobe C4. The valvula covers its dorsal surface (Fig. 1A) (Bell and Szabo 1986; Nieuwenhuys and Nicholson 1969a).

The posterior caudal lobe lacks the laminar organization, characteristic of the central lobes and valvula. Purkinje cells are randomly scattered in the molecular zone (Bell and Szabo 1986; Campbell et al. 2007). Based on Nissl-stained material and material stained with an antibody to the IP3 receptor, the posterior caudal lobe is divided into three zones: dorsal, medial, and ventral zones, and each zone contains a relatively isolated group of Purkinje cells (Campbell et al. 2007). Climbing fibers terminate on the soma and smooth proximal dendrites of Purkinje cell, and parallel fibers terminate on the spiny distal dendrites. Purkinje cell axons end locally on efferent cells, which in turn project outside the lobe to the mesencephalon. Efferent cells also receive input from parallel fibers.
(Campbell et al. 2007). Thus the local circuitry in the posterior caudal lobe (Fig. 2B) is roughly similar to that in the central lobes (Han et al. 2006) and the valvula (Zhang et al. 2006).

Based on our morphological and physiological observations, four groups of cells can be recognized in the parasagittal plane. The first group of cells are multipolar Purkinje cells, which have three or more primary dendrites and are scattered in the center area of the lobe (Fig. 1, B and D). Multipolar Purkinje cells are equivalent to the medial and ventral Purkinje cells of Campbell et al. (2007). The second group of cells are fan-shaped Purkinje cells, the cell bodies of which are restricted in a small band in the dorsal cap of the lobe (Fig. 1, B and C), with a fan-shaped dendritic tree toward the dorsal surface of the lobe. Fan-shaped Purkinje cells are equivalent to the dorsal Purkinje cells of Campbell et al. (2007). The third group of cells are small Purkinje cells, located in a narrow band beneath the fiber tract entering the lobe (Fig. 1B, sm c). These Purkinje cells were not distinguished as a separate group by Campbell et al. (2007). The final group of cells are efferent cells, a group of small cells located in a small area dorsal to the fiber tract (Fig. 1B, ef c). The efferent cells we recorded are probably a subset of the efferent cell population labeled with retrograde tracers by Campbell et al. (2007). The physiology, as well as the morphology of these cell types are described in the following text.

**Purkinje cells**

A total of 138 cells were classified as Purkinje cells. Under voltage clamp, the averaged \( (n = 21) \) access resistance was 21.8 ± 5.2 MΩ, the cell input resistance was 17.4 ± 8.5 MΩ, and the whole cell capacitance was 1.3 ± 0.5 nF. The initial resting membrane potentials ranged between –62 and –82 mV \( (n = 83) \) under current clamp. The subsequent morphological identification further classified these cells into three subtypes—multipolar Purkinje cell \( (n = 104) \), fan-shaped Purkinje cell \( (n = 14) \), and small Purkinje cell \( (n = 20) \). Although all fan-shaped and small Purkinje cells were successfully labeled, 51 of 104 cells were morphologically identified as multipolar Purkinje cells, and others were classified based on their location in the lobe and physiological properties similar to the morphologically identified cells. The physiology and morphology of these cells are described in the following sections.

**MULTIPOLAR AND FAN-SHAPED PURKINJE CELLS.** Purkinje cells in the central lobes and the valvula fire three types of spikes: a small narrow sodium spike, a large broad sodium spike, and a large broad calcium spike (Han and Bell 2003; Zhang et al. 2006). In contrast, the majority of morphologically identified Purkinje cells in the posterior caudal lobe fire only one type of spike, a large narrow spike (1–2 ms in duration, 40–60 mV in amplitude), in response to a voltage or current step injected into the soma. Figure 3A shows an example of a morphologically identified multipolar Purkinje cell that fired a sequence of large narrow spikes in response to a current step of 300 pA (bottom trace) or higher. The cell fired repetitively with little frequency adaptation when current step of 700 pA were injected (top trace). No broad spikes were observed regardless of the intensity of the injected current. The firing frequency of 12 cells to somatic current steps are summarized in Fig. 3B, indicating that most of these cells do not fire action potentials until current intensity reach 300 pA. The amplitudes of these spikes decreased markedly during the repetitive firing when more intense current steps were injected (Fig. 3A, bottom panel). Such spike amplitude changes were observed in nearly all Purkinje cells in this study. Similar changes in the ampli-

**FIG. 2.** Experimental arrangements. A: drawing of a multipolar Purkinje cell labeled with biocytin in the central area of the lobe and a schematic drawing of a single climbing fiber, oriented in the sagittal plane. The climbing fiber (shown in red) terminates only on the soma and smooth proximal dendrites of the Purkinje cell. Axon (arrow, shown in green) is simplified. Stimulus electrodes are placed in the distal molecular layer (SM) and adjacent area (SA) to the soma to activate parallel fibers and climbing fiber, respectively. Rec, recording electrode. B: diagram of local circuitry of the posterior caudal lobe in the transverse plane. Some essential features of local circuitry are shown: the climbing fiber terminates on the soma and proximal dendrites of a multipolar Purkinje cell. The Purkinje cell terminates locally on the efferent cell. Parallel fibers, arising from EGp in both sides, excite the molecular layer dendrites of Purkinje cells, as well as the dendrites of efferent cell, stellate cell, and Golgi cell. Inhibitory neurons are shown in gray and excitatory neurons in white. cf, climbing fiber; EGp, eminientia granularis posterior; Go c, Golgi cell; gr c, granule cell; GrL, granular layer; IO, inferior olive; mf, mossy fiber; ML, molecular layer; Pc, Purkinje cell; pf, parallel fiber.
Purkinje cells and fan-shaped Purkinje cells. The primary criteria for classifying these cells as Purkinje cell are their spiny dendritic arbors and locally terminating axons (Campbell et al. 2007; Nieuwenhuys and Nicholson 1969a). The criteria for further classification of these cells are described in the following text.

Multipolar Purkinje cells were located in the central portion of the molecular zone of the lobe with three or more smooth dendrites arising from the soma in random directions. Dendrites branched at variable distances from the soma and then became spiny and branched further. The irregular branching magnitude of narrow sodium spikes during repetitive firing were observed in Purkinje cells of the central lobes and the valvula (Han and Bell 2003; Zhang et al. 2006).

Pharmacological methods were used to show that the large narrow spikes are sodium spikes. Bath application of the calcium channel blocker, cadmium (100–200 μM), had no effect whereas (Fig. 4A, middle trace), subsequent addition of the sodium channel blocker, TTX (1 μM; n = 4), eliminated all spikes (Fig. 4A, bottom trace). If TTX was given first, the spikes disappeared (Fig. 4B, bottom trace; n = 3).

The subsequent morphological identification showed that the Purkinje cells with the preceding physiological and pharmacological characteristics fell into two groups: multipolar
pattern of these cells is in striking contrast to the palisade pattern of Purkinje cell dendritic arbor in the central lobes and the valvula where the spiny dendrites rise in parallel from the ganglionic layer to the outer surface of the molecular layer (Campbell et al. 2007; Han et al. 2006; Meek 1992). The axons of multipolar Purkinje cells arise from the soma or one of the main dendrites, branch within a short distance from soma, and terminate locally. Examples of multipolar Purkinje cells are shown in Figs. 5, A and B, and 6B. In some cases, the axons ended abruptly at the surface of the slices where it had been cut during slice preparation (Fig. 6B).

The fan-shaped Purkinje cells are distinguishable from multipolar Purkinje cells primarily based on the relatively parallel organization of their dendritic arbor and by their location within the caudal lobe. They occupy a densely packed cell body band in the dorsal cap. As shown in Fig. 5, C–E, fan-shaped Purkinje cells typically have three or more smooth primary dendrites, some of the primary dendrites run above the cell body band and branch repeatedly at relatively regular distance. These tertiary dendrites are relatively short but run parallel toward the dorsal surface of the lobe, forming a fan-shaped dendritic tree in the sagittal plane, whereas other dendritic arbors extend toward the deep of the lobe with less branching. The relatively orderly arrangement of their cell bodies and the regularity of their dendrites thus form a laminar region in the lobe (Campbell et al. 2007). The axons of fan-shaped Purkinje cells also terminate locally (Fig. 5D).

SMALL PURKINJE CELLS. We found a small number of Purkinje cells that fired two distinct spike types in response to intracellular current steps: a small narrow spike and a large broad spike. These cells have smaller cell bodies and were restricted to a small area in the lobe (Fig. 1B). One such cell is shown in Fig. 4C. This cell responded to current steps at 200 and 400 pA with only large narrow spikes (top and middle traces, respectively), whereas the cell did respond to a 600-pA step with a small narrow, early large broad and late large broad spikes.
Similar to other two subtypes of Purkinje cells, voltage-dependent narrow spike amplitude change occurred when intracellularly injected current step intensity changed (Fig. 4C, bottom trace). These results are consistent with our recent observations of Purkinje cells in the central lobes and the valvula of the mormyrid cerebellum (Han and Bell 2003; Zhang et al. 2006).

Pharmacological methods were used to show that the narrow spikes are sodium spikes and that the broad spikes are calcium spikes. Bath application of TTX (1 μM), eliminated narrow spikes and left the later broad spike unaffected (Fig. 4D, middle trace). Subsequent addition of cadmium (200 μM), eliminated the late broad spikes (Fig. 4D, bottom trace). This sequence of events was observed in five Purkinje cells. If cadmium was given first, the late broad spikes disappeared but the narrow spikes remained. Subsequent addition of TTX eliminated narrow spikes (not shown). This sequence of events was observed in three cells.

Subsequent morphological results showed that all cells (n = 20) that fire both narrow and broad spikes have a smaller cell body (<7 μm in diameter vs. 10–15 μm for fan-shaped cells and 10–20 μm for multipolar cells), and smaller dendritic arbor. Unlike either multipolar or fan-shaped Purkinje cells, small Purkinje cells typically have one (2 in some cases) smooth primary dendrite, which become spiny and branch randomly. The axons of small Purkinje cells, if revealed, end locally. One such small Purkinje cell is shown in Fig. 5F, another reconstructed such cell is shown in Fig. 6A, showing locally ended axon. The morphology of small Purkinje cell again is different from Purkinje cells in the central lobes and the valvula (Nieuwenhuys and Nicholson 1969a), although the spike types it fires are similar to its counterparts in the central lobes and the valvula as presented in the preceding text.

The preceding results indicate that the majority of Purkinje cells in the posterior caudal lobe of the mormyrid cerebellum fire only one type of spike, large narrow sodium spike; whereas a small portion of the Purkinje cells fire at least two types of spikes—a narrow sodium spike, and a broad calcium spike as shown in the central lobes and valvula (Han and Bell 2003; Zhang et al. 2006) and mammalian cerebellum (Hounsgaard and Midtgaard 1988; Linas and Sugimori 1980; Stuart and Hausser 1994). The small sodium spikes have the lower threshold to intracellular current pulses, whereas the broad calcium spikes have a higher threshold.

RESPONSES TO PARALLEL FIBER STIMULATION. Responses of Purkinje cells evoked by parallel fiber stimulation in mammalian cerebellum are mediated entirely by glutamate receptors of the AMPA type and do not include any NMDA receptor component (Konnerth et al. 1990). The same is true of parallel fiber-evoked synaptic potentials in mormyrid posterior caudal lobe Purkinje cells. The most easily tested cells are the large multipolar Purkinje cells. The amplitudes and durations of EPSPs evoked by parallel fiber stimulation were 1–5 mV and 20–65 ms, respectively. The latencies were 9.5 ± 1.8 ms, and the time constants of decay were 38.8 ± 2.4 ms (90–10%; n = 21). The latencies and time constants of decay of the parallel fiber evoked EPSCs were 8.1 ± 0.9 and 34.2 ± 4.5 ms, respectively (n = 19). Both EPSPs (Fig. 7A) and EPSCs (Fig. 7B) showed clear paired-pulse facilitation when pairs of stimuli were delivered between 10 and 200 ms as occurs also in mammals (Eccles et al. 1967; Konnerth et al. 1990). Although the strongest facilitation occur between 20 and 100 ms of interpulse intervals, the facilitation lasts 400–500 ms in most cases. One of such examples is illustrated in Fig. 7C, and similarly pooled data (n = 7) are shown in Fig. 7D. The EPSCs...
or EPSPs evoked by parallel fiber stimulation in multipolar Purkinje cells were not affected by glutamate NMDA type receptor antagonist AP5, but were completely blocked by the AMPA antagonist CNQX (Fig. 8A; 4 of 4 cells). No additional synaptic current was observed under CNQX in cells recorded under voltage clamp when the membrane potential was held at 0 mV (3 of 3 cells, Fig. 8B). This indicates the absence of NMDA receptor-mediated transmission because the magnesium block of NMDA receptors would be removed at this potential.

We next tested the parallel fiber responses in the fan-shape Purkinje cells (n = 4) and small Purkinje cells (n = 3). Similar results were observed, the parallel-fiber-evoked EPSCs or EPSPs also showed paired-pulse facilitation. These responses again were not affected by AP5 but were completely blocked by CNQX, and no additional synaptic responses were observed when the holding potential was changed from −70 to 0 mV (Fig. 8C, D and D).

These results indicate that the parallel fiber responses in all three subtypes of Purkinje cells are similar, mediated entirely by AMPA type glutamate receptors. We did not observe any inhibitory postsynaptic current or potential (IPSC or IPSP) in either type of Purkinje cells when extracellular stimuli were applied.

**RESPONSES TO CLIMBING FIBER STIMULATION.** Climbing fiber activation evoke complex spike under current clamp and large all-or-none EPSP under voltage clamp in mammalian Purkinje cells (de Ruiter et al. 2006; Eccles et al. 1967; Konnerth et al. 1990). Large all-or-none EPSC and EPSP were obtained from Purkinje cells in the central lobes and valvula of mormyrid cerebellum under voltage or current clamp (de Ruiter et al. 2006; Han and Bell 2003; Zhang et al. 2006). However, such all-or-none response was recorded only in some of Purkinje cells in the posterior caudal lobe by placing stimulus electrode around the recorded cell bodies. The subsequent morphological results showed that all cells that had climbing fiber response in all-or-none fashion were multipolar Purkinje cells (n = 16). Similar to Purkinje cells in the central lobes and the valvula, climbing fiber response in the posterior caudal lobe under current clamp is a large all-or-none EPSP of 40–50 mV, consisting of two distinct phases, a large initial phase lasting ~10 ms followed by a second phase that consisted of a slow return to baseline and lasted ~60–100 ms (Fig. 9A). The latencies were 5.4 ± 0.9 ms, and the time constants of the EPSP decay (90–10%) were 44.3 ± 5.5 ms (n = 11). Interestingly, a large broad spike was sometimes evoked during the initial phase (Fig. 9, A, B, and D), although these cells normally do not fire broad spikes in responding to somatic depolarization current pulse injection described earlier. The large all-or-none EPSCs recorded under voltage clamp also have two phases and lasted for ~40–50 ms, and a secondary large broad spike can be seen sometimes (Fig. 9D). Similar attempts were made to examine all-or-none responses in fan-shaped and small Purkinje cells, and no such responses were observed (n = 15).

The synaptic response to climbing fiber stimulation showed here is similar to mammalian climbing fiber responses in showing a moderate paired-pulse depression and in being mediated exclusively by AMPA receptors (Hashimoto and Kano 1998; Konnerth et al. 1990; Schmolesky et al. 2003). The largest depression normally occurred between 20 and 50 ms of interpulse interval (Fig. 9A), gradually recovering to baseline when the interval increased to 200 ms. An example of such changes under current clamp is shown in Fig. 9B; pooled data of six cells are shown in Fig. 9C. In comparison, the recovery from the paired-pulse depression in the mormyrid caudal lobe is faster than in the mammalian cerebellum; the latter recovery takes >5 s (Konnerth et al. 1990). The climbing fiber EPSC was not affected by NMDA receptor antagonist AP5 but was
either completely abolished or reduced them to near the baseline by additional AMPA receptor antagonist CNQX (Fig. 9D; \( n = 5 \)). Mediation by glutamate receptors of the AMPA type was also shown by the absence of any response under CNQX even at the holding membrane potential of 0 mV where the magnesium block on NMDA receptors would be removed (Fig. 9E; \( n = 3 \)).

**Efferent cells**

Recording from efferent cells in the posterior caudal lobe was initially quite a challenge due to their smaller number (ratio of efferent cells to Purkinje cells is 1:25, estimated by Campbell et al. 2007) and smaller cell body size (\( \sim 6-8 \mu m \) in diameter vs. 10–20 \( \mu m \) for Purkinje cells). Finding of these cells being restricted in a small area (Fig. 1B) led us successfully to record from 37 cells that showed similar physiological properties but differing from Purkinje cells. Among these cells, 17 were subsequently morphologically identified as efferent cell (see following text). The resting membrane potentials of efferent cells ranged from \( 55 \) to \( 78 \) mV (\( n = 21 \)). Efferent cells fire a single type of spike, a large narrow spike with a prominent afterhyperpolarization, similar to the spikes in efferent cells in the central lobes and the valvula and many other CNS neurons (Fig. 1A, top trace). The efferent cells fired repetitively to intracellular current injection with little if any adaptation in frequency and amplitude (Fig. 1A, bottom trace). Thus the spikes efferent cell fires are distinguishable...

**FIG. 9. Responses of multipolar Purkinje cells to climbing fiber activation.** The recordings were performed under either voltage (hold potential, \( -70 \) mV) or current clamp. Stimulus electrodes were placed at adjacent area (SA, <50 \( \mu m \)) to the somas patched. A: single sweeps of climbing fiber responses were superimposed. Under current clamp, Purkinje cells respond to stimulation near the recorded soma with a large, all-or-none EPSP, consisting of a large, brief initial phase and a slow ramp-like return to the baseline. Pairs of stimuli separated by 40 or 50 ms were delivered to test the paired-pulse interaction. Stimuli were near threshold (5–15 \( \mu A \) in most cases) for a climbing fiber EPSP. The EPSP to the 2nd stimulus was reduced (paired-pulse depression) when the 1st stimulus evoked an EPSP (black trace). The second EPSP was of full size when the 1st stimulus failed to evoke a response (gray trace). A secondary broad spike sometimes follows the initial EPSP. B: averaged responses of a multipolar Purkinje cell tested in a series of interpulse intervals under current clamp. Subtractions were performed in the same way as for paired-pulse facilitation of parallel fiber responses. C: graph of magnitude of paired-pulse depression as a function of interpulse intervals. The peak values of the initial phase of EPSPs were taken from 6 Purkinje cells and averaged. D: pharmacology of climbing fiber responses (single sweeps superimposed). Similar to the recordings under current clamp, activation of climbing fiber under voltage clamp evokes a large, all-or-none EPSC, consisting of a large, brief initial phase and a slow ramp-like return to the baseline. In this cell, each of 2 stimulus pulses evokes an all-or-none EPSP, that shows a moderate paired-pulse depression (top traces). The 1st stimulus also evokes a broad spike. The climbing fiber EPSC was not affected by the NMDA receptor antagonist AP5 (middle traces) but is completely eliminated by AMPA receptor antagonist, CNQX (bottom traces). E: averaged responses of another Purkinje cell to climbing fiber activation under voltage clamp. The all-or-none EPSP was completely blocked by CNQX, and no response remains when the hold potential was at 0 mV. The response was partially recovered after washout.
from that multipolar Purkinje cell and fan-shaped Purkinje cell fire; the latter show clear amplitude adaptation as presented earlier. The spiking threshold of efferent cells are also different from that of Purkinje cells. In most cells tested, they fire action potentials to somatic current steps of as low as 20–30 pA at a resting membrane potential of −70 mV. The relation of spiking frequency and current intensities are illustrated in Fig. 3D. The pooled data (n = 8) shown in Fig. 3D indicate that the spiking threshold in efferent cells are significantly lower than that in Purkinje cells (P < 0.001). Further tests indicate that efferent cell spikes were not affected by bath application of cadmium, but reversibly abolished by TTX, indicating a sodium spike (Fig. 10B; n = 6).

Parallel fiber stimulation evoked EPSCs or EPSPs that showed a moderate paired-pulse facilitation (Fig. 11, A and B, top traces). The EPSC latencies were 7.9 ± 1.2 ms (P > 0.05, compared with that of Purkinje cells) and the time constant of decay were 26.5 ± 3.4 ms (n = 9; P < 0.05, compared with that of Purkinje cells). The parallel fiber EPSCs were completely blocked or reduced to near the baseline by CNQX in four cells tested (Fig. 11A, middle trace). No additional response was seen when the membrane potential was held at 0 mV (Fig. 11A, bottom trace). If AP5 was bath applied first, the synaptic EPSCs were not affected (Fig. 11B, 2nd trace), and additional CNQX to the bath completely blocked the responses (Fig. 11B, 3rd trace). When the membrane potential was held at 0 mV, a small brief outward current was seen (Fig. 11B, bottom trace). Such tests were carried out in two cells.

We were not able to evoke large all-or-none EPSPs in efferent cells by placing stimulus electrode around the patched cell body, suggesting that the efferent cells do not receive climbing fiber input, similar to efferent cells in the central lobes and the valvula. We did not observe clear IPSP or IPSC from any efferent cells when extracellular stimuli were applied.

The morphology of efferent cells is clearly distinguishable from Purkinje cells. Efferent cell dendrites are fewer in number and lack dendritic spines. Two examples of efferent cells labeled by biocytin are shown in Fig. 12. Typically, one (sometimes 2) primary dendrite arises from the soma, giving off a few fine smooth secondary branches. The distal dendritic branches are usually fine and beaded. The area covered by a single efferent cell dendritic tree is smaller than that of multipolar and fan-shaped Purkinje cells in the caudal lobe. In several cases, a short process with a ball ending, probably a cut axon, was seen from the soma (Fig. 12B, arrow) and no local axon terminals observed. The morphology of our efferent cells is similar to that of one subtype of efferent cells labeled in vivo (Campbell et al. 2007).

**DISCUSSION**

The posterior caudal lobe concerned in the present study was referred as the molecular zone of the caudal lobe (Bell and Szabo 1986) and is functionally associated with electrosense (Bell et al. 1992; Campbell and Bell 2003). Cells in this lobe include three groups of Purkinje cell, eurydendroid efferent cell, and stellate cell; its inputs include parallel fibers from the adjacent EGp and climbing fiber from the inferior olive (Bell and Szabo 1986; Campbell et al. 2007). The present study confirmed and extended these previous studies by providing new physiological information for the cells in the lobe and by classifying Purkinje cells into three subtypes based on our physiological and morphological results.

The posterior caudal lobe differs from other cerebellar structures, the central lobes and the valvula, by lacking a clear laminar organization (Han et al. 2006; Meek and Nieuwenhuys...
The recent morphological study by Campbell et al. (2007) and present physiological results, however, indicate that there are many similarities as well as differences between the posterior caudal lobe and other cerebellar structures.

As in other cerebellar structures, cells in the posterior caudal lobe respond to parallel fiber input with glutamate AMPA-type receptor-mediated synaptic transmission and an all-or-none response to climbing fiber input, which is also entirely mediated by AMPA-type glutamate receptors. These results are consistent with our observations from the central lobe and the valvula (Han and Bell 2003; Zhang et al. 2006). The similarities also include a small narrow sodium spike and a large broad calcium spike small Purkinje cell fires in response to intracellular current steps. In addition, responses of efferent cells are also similar to those observed in the central lobes and the valvula.

The differences, however, are also evident. First, multipolar and fan-shaped Purkinje cells in the posterior caudal lobe fire only large narrow sodium spike, whereas Purkinje cells in other cerebellar structures of mormyrid fish fire both small sodium and large calcium spikes (Han and Bell 2003; Zhang et al. 2006). Second, it is unexpected that fan-shaped and small Purkinje cells in the posterior caudal lobe lack all-or-none climbing fiber response because our immunohistochemical materials (J. Meek, V. Z. Han, and C. C. Bell, unpublished data) shows that climbing fibers stained by antibodies against calretinin and calbindin are distributed in both the central area of the lobe, where multipolar Purkinje cells are located, and in the dorsal cap of the lobe where fan-shaped Purkinje cells are located. The patterns of the labeled climbing fiber terminals, however, appear to differ between the two regions. In the central area, individual terminals wrap the cell bodies and proximal dendrites back and forth with tens of buttons, forming outlines of single multipolar cells; whereas in the dorsal cap, labeled terminals appear to be restricted in the cell body band.

It is anticipated that in vivo recordings could help determine whether these cells exhibit all-or-none climbing fiber responses and whether their climbing fiber responses differ from those observed in multipolar Purkinje cells. Nevertheless, climbing fiber and parallel fiber inputs are important components in both the mammalian cerebellum and mormyrid cerebellum. The multipolar Purkinje cell in the posterior caudal lobe, therefore appears to be a suitable model to understand how these two input systems interact during information processing in the cerebellum in general.

Second, inhibition in the valvula is prominent. Small EPSPs can be marked enhanced or an IPSP can be reversed to EPSP when GABA<sub>A</sub> receptor antagonist bicuculine was bath applied in both Purkinje cells and efferent cells (Zhang et al. 2006). However, we have been unable to record any IPSPs from Purkinje cells or efferent cells in the posterior caudal lobe in response to parallel fiber stimulation. These results are consistent with the immunohistochemical materials, showing that inhibitory interneuron stellate cells are rare in the posterior caudal lobe but numerous in the valvula and the central lobes (Han et al. 2006; Campbell et al. 2007; Meek et al., unpublished data). Thus Purkinje cells with locally ended axons are the major source of inhibition in the posterior causal lobe.

Another difference between our results and previous study is the distribution of efferent cells in the posterior caudal lobe. Using retrograde labeling method, it showed that efferent cells are mainly distributed in the border between the posterior caudal lobe and the EGp, with a relatively scattered pattern (Campbell et al. 2007); whereas efferent cells labeled intracellularly by whole cell patch method in the present study are restricted in a small area (Fig. 1B). It is likely that only some of efferent cells can be sampled by visualized patch method in the present study. The morphology of our efferent cells are similar to one of the effenter cells of previous study (Fig. 7A).
of Campbell et al. 2007), but none of our efferent cells fits eurydendroid cell type (Meek 1992; Meek and Nieuwenhuys 1991).

In summary, the present study shows that basic physiology of cells in the posterior caudal lobe are largely similar to that of cells in other cerebellar structures of mormyrid fish, as well in mammalian cerebellum (Ito 1984). These similarities suggest that what is learned in the mormyrid cerebellum may be a source of hypotheses regarding cerebellar function in mammals. Some of the differences or unique features of the mormyrid cerebellum make it suitable for investigating some aspects of cerebellar function, which cannot be easily addressed in the mammalian cerebellum. In particular, scattered large multipolar Purkinje cells and their locally ending axon terminals in the center area of the posterior caudal lobe indicate that this cell type could be a suitable site for investigating effects of Purkinje cell on its target cells using dual cell recording method.

ACKNOWLEDGMENTS

The authors thank Drs. Nathaniel Sawtell, Curtis Bell, and Jian-Tian Qiao for helpful discussion and comments on manuscript.

GRANTS

The work is supported by National Institute of Neurological Disorders and Stroke Grant NS-44061 to V. Z. Han.

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